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VOLUME 29 MAY, 1951 NUMBER 5

THE STRUCTURE OF SEDAMINE[1]

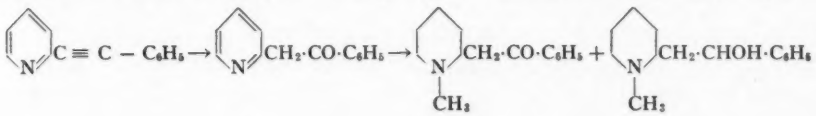
BY LÉO MARION, ROBERT LAVIGNE, AND LIONEL LEMAY

Abstract

The infrared absorption spectrum of sedamine, an alkaloid occurring in *Sedum acre* L., is exactly superposable on that of synthetic 1-methyl-2-(β-hydroxy-β-phenylethyl)-piperidine. The melting points of the bases and their hydrochlorides, alone and in admixture, indicate that sedamine is probably one of the optically active forms of the synthetic diastereomer.

The alkaloid sedamine was found in *Sedum acre* L., by Kolesnikov and Shvartsman (3) who assigned to it the empirical formula $C_{17}H_{24}O_2N$. Later it was isolated again from the same source by one of us (4) who found that it is accompanied by nicotine and who corrected the formula to $C_{14}H_{21}ON$. An investigation of the species *Sedum sarmentosum* Bunge had shown that this plant contained two alkaloids, i.e., N-methylisopelletierine and the corresponding dihydro-base, 1-methyl-2-(β-hydroxypropyl)-piperidine, the latter being one of the optically active isomers of that structure (5). The occurrence of such bases in another species of *Sedum*, together with an examination of the empirical formula of sedamine, prompted the assumption that sedamine might be an N-methylpiperidine carrying in the 2-position a β-hydroxy-β-phenylethyl group (IV). The correctness of this assumption has now been established by the synthesis of the base IV and the direct comparison of this base with sedamine.

The synthesis was carried out with the condensation product of α-picoline and benzaldehyde as starting material, following the procedure outlined by Scheuing and Winterhalder in connection with their synthesis of lobeline (6). The product was treated with bromine and the adduct converted to 1-α-pyridyl-2-phenylacetylene (I) which by the action of concentrated sulphuric acid gave rise to α-phenacylpyridine (II). Howton and Golding (1) report that the platinum oxide-catalyzed hydrogenation of the methobromide of II gives



I II III IV

[1] *Manuscript received January 19, 1951.*
Contribution from L'Institut de Chimie, Université de Montréal, Montreal, Que., and the Division of Chemistry, National Research Council, Ottawa, Canada. Issued as N.R.C. No. 2365.

a good yield of 1-methyl-2-phenacylpiperidine (III), but that further hydrogenation yields a compound in which they tentatively assume that the phenyl group has been reduced to a cyclohexyl group. In the present instance, however, the metho-*p*-toluenesulphonate of II was prepared and the platinum oxide-catalyzed reduction of this salt was found to give a mixture in which the main component, 1-methyl-2-phenacylpiperidine (III), was accompanied by an appreciable quantity of the desired product, 1-methyl-2-(β-hydroxy-β-phenylethyl)-piperidine (IV). A compound of this structure contains two asymmetric C atoms and must, therefore, exist in two diastereoisomeric pairs. From it was obtained a crystalline fraction melting at 88°C.

Recrystallization of sedamine did not alter the melting point (88–89°C.), but reanalysis of the alkaloid showed the formula to be $C_{14}H_{21}ON$, without the third of a mole of water of crystallization reported earlier (4). The melting point of sedamine was not visibly depressed by admixture with the synthetic base (IV). However, while sedamine hydrochloride melted at 205°C., the hydrochloride of synthetic IV melted at 185°C. as also did the mixture of the two salts. Nevertheless, the infrared absorption spectra of the two bases (Fig. 1) are superposable and, therefore, the bases must be identical. The discrepancy in the melting points of the hydrochlorides is likely attributable to the fact that sedamine is presumably optically active—although insufficient material is available for the determination—while the synthetic base is racemic.

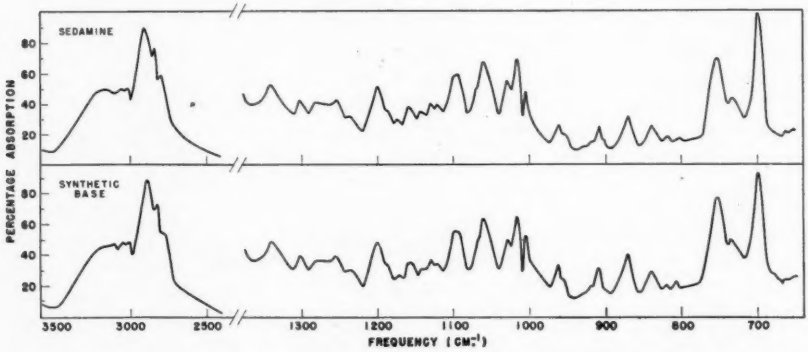


FIG. 1. *Infrared spectra of sedamine and the synthetic base, measured on a Perkin–Elmer 12B single beam spectrometer with sodium chloride prism, 1 mm. cell. The alkaloids were in carbon disulphide solution, 10 mgm. per ml.*

Amongst the minor alkaloids of *Lobelia inflata* L., Wieland and his coworkers (7) have reported two isomeric bases of formula $C_{14}H_{21}ON$, one melting at 103°C. the other at 81°C. The latter when oxidized gave rise to α-N-methylpiperidyl-carboxylic acid and was tentatively assigned structure IV which has now been shown to be that of sedamine. It is impossible to state whether the lower melting of the two *Lobelia* alkaloids is identical either with sedamine or its optical antipode or whether it is one of the other diastereoisomeric pair.

Experimental*

Sedamine

The analytical figures reported previously (4) for this alkaloid agreed with the formula $C_{14}H_{21}ON.1/3H_2O$. Sedamine was recrystallized from petroleum ether and this did not alter its melting point, 88–89°C. It was dried in an evacuated desiccator before analysis. Calc. for $C_{14}H_{21}ON$: C, 76.68; H, 9.65; N, 6.39%. Found: C, 76.46; H, 9. 46; N, 6.28%.

α-Phenacylpyridine

1-α-Pyridyl-2-phenylacetylene was prepared by the method of Scheuing and Winterhalder (6). A quantity (31.6 gm.) of this substance was added to water (71 cc.) and concentrated sulphuric acid (64.5 cc.) and the mixture heated for 20 min. on the steam bath. After cooling, water (64.5 cc.) was added, the solution was alkalized with ammonia and extracted with ether. The ether extract was dried over sodium chloride and distilled on the steam bath to remove the solvent. The residual base after distillation, b.p. 130–135°C. at 0.6 mm., consisted of a yellow oil that solidified on cooling to yellow needles, m.p. 51°C. After sublimation and recrystallization from ether–hexane, it melted at 58–59°C. The literature gives m.p. 54°C. (2) and 59.1–60.7°C. (1). Yield, 29.7 gm. (85.5%). Calc. for $C_{13}H_{11}ON$: C, 79.18; H, 5.58%. Found: C, 79.29, 79.30; H, 5.41, 5.39%. The oxime melted at 117–118°C. (the literature records m.p. 116°(1)).

α-Phenacylpyridine metho-p-toluenesulphonate

To a solution of α-phenacylpyridine (17.3 gm.) in benzene (32 cc.), methyl *p*-toluenesulphonate (26.2 gm.) was added and the resulting solution heated on the steam bath for one hour. After a few minutes, the solution turned red and crystals separated. After one hour benzene (100 cc.) was added, the mixture allowed to cool and filtered. The filtered crystalline product, after recrystallization from methanol–ether, consisted of pale yellow prisms, m.p. 149–150°C. Weight, 21.8 gm. Calc. for $C_{21}H_{21}O_4NS$: C, 65.85; H. 5.48; N, 3.60. Found: C, 65.85, 65.70; H, 5.33, 5.37; N, 3.64, 3.58%.

α-Phenacyl N-methylpiperidine

α-Phenacylpyridine metho-*p*-toluenesulphonate (17.3 gm.) was dissolved in glacial acetic acid (170 cc.) and hydrogenated for 30 hr. in the presence of platinum oxide catalyst (0.2 gm.) at 50 lb. pressure. The catalyst was filtered and the filtrate distilled under reduced pressure on the steam bath. There remained a residue that was dissolved in water (150 cc.), the solution was alkalized with sodium hydroxide and extracted with ether. The combined ether extract was dried over sodium sulphate and evaporated to dryness. A pale yellow oil remained that boiled at 125–130° at 0.2 mm.; wt. 9.27 gm.

* *All melting points are corrected.*

The oily product (9.27 gm.) was dissolved in hot methanol (50 cc.) and added to a hot solution of picric acid (13 gm.) in methanol (100 cc.). Boiling was continued for 15 min. and the solution allowed to cool. A crystalline picrate separated (10.43 gm.) that, after recrystallization from boiling methanol, consisted of bright yellow aggregates, m.p. 159–160°C. (The literature (1) reports 159.2–159.4°C. as the melting point.) Calc. for $C_{20}H_{22}O_8N_4$: C, 53.81; H, 4.93; N, 12.56%. Found: C, 53.63, 53.81; H, 4.92, 5.03; N, 12.38, 12.41%. The base recovered from the pure picrate was an oil.

The picrate (2 gm.) was decomposed by shaking with dilute hydrochloric acid and ether. The aqueous solution was neutralized with potassium carbonate and extracted repeatedly with ether. The combined extract was dried over sodium sulphate and distilled on the steam bath. A yellow oil was left that was distilled *in vacuo*, b.p. 125–130°C. at 0.6–0.8 mm.; wt., 0.837 gm. The distillate was dissolved in absolute ether and the solution saturated with hydrogen chloride. A white gum separated that was crystallized from ethanol–acetone and recrystallized several times from ethanol–ether. The hydrochloride consisted of small colorless prisms, m.p. 166–167°C. Calc. for $C_{14}H_{19}ON \cdot HCl$: C, 66.26; H, 7.94; N, 5.40%. Found: C, 66.36; 66.11; H, 8.04, 7.80; N, 5.50%.

1-Methyl-2-(β-hydroxy-β-phenylethyl)-piperidine

The methanolic mother liquor from which the picrate of α-phenacyl-N-methylpiperidine had first separated was diluted with water, the solution made strongly acid with hydrochloric acid, and repeatedly extracted with ether to remove the liberated picric acid. The aqueous solution was then alkalized with sodium hydroxide and extracted with ether. This ether extract was dried over sodium sulphate and evaporated to dryness. There remained an oily residue that was distilled *in vacuo*, b.p. 165–170°C. at 1.7–2 mm.; wt. 3.38 gm. On standing, the distillate partially crystallized; it was dissolved with warming in petroleum ether and the solution on cooling deposited a crop of crystals. After several recrystallizations from petroleum ether the product was obtained as colorless elongated plates, m.p. 88°C. Calc. for $C_{14}H_{21}ON$: C, 76.68; H, 9.65; N, 6.39%. Found: C, 76.57, 76.79; H, 9.33, 9.48; N, 6.29, 6.40%. The infrared absorption spectrum of this compound reveals the presence of a hydroxyl group but shows no absorption in the carbonyl region. The total spectrum (Fig. 1) is superposable on that of sedamine.

A small quantity of the base was dissolved in methanol and neutralized to Congo red by the dropwise addition of methanol containing concentrated hydrochloric acid. The solution was then evaporated on the steam bath, the residue dissolved in methanol and the solution again evaporated to dryness. The final residue was dissolved in a few drops of methanol and the cooled solution diluted with absolute ether to incipient turbidity. Inoculation with a small crystal of sedamine hydrochloride initiated crystallization. The salt was recrystallized twice from methanol–ether from which it separated as colorless needles, m.p. 185°C. In admixture with sedamine hydrochloride it melted at 183°C.

Acknowledgment

The absorption spectra were taken by Dr. R. N. Jones and Mr. R. Lauzon, National Research Council, whose courtesy is gratefully acknowledged. Our thanks are also due to the Office de Recherches Scientifiques de la Province de Québec, for the award of a Fellowship to one of us (R.L.).

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THE STRUCTURE OF JABORANDINE, THE ALKALOID OF *PIPER JABORANDI* VELLOSO. I.[1]

By K. Wiesner

Abstract

Jaborandine, the active principle of *Piper jaborandi* Velloso, has been shown to be an ester of *p*-aminobenzoic acid and an amino alcohol which contains a tertiary nitrogen with an $N-CH_3$ group and has the formula $C_{11}H_{23}ON$.

The Brazilian plant *Piper jaborandi* used as a local anaesthetic in popular medicine has been shown by Oscar Ribeiro and Antenor Machado (1) to contain an alkaloid which is responsible for the anaesthetic properties of the plant. We have had the opportunity of examining a small amount of the alkaloid, and we present our first results in this communication. The alkaloid forms a crystalline hydrochloride which melts at 134°C., resolidifies, and remelts at 154–156°C. A picrolonate, m.p. 148–149°C., was also prepared. The base itself can be distilled in high vacuum and solidifies to a low melting crystalline solid. The analyses of these derivatives agree with the formula for the base $C_{18}H_{28-30}O_2N_2$, some of the analyses seemingly agreeing better with the H_{30} than with the H_{28} formula. The base forms salts with one equivalent of acid and contains one $N-CH_3$ group. The result of a potentiometric microtitration showed only one basic nitrogen with the dissociation constant pK (cellosolve) = 8.14. The second nitrogen must therefore be very weakly basic or nonbasic. The ultraviolet spectrum in alcohol shows a maximum at 294 mμ ($\log \epsilon = 4.52$). As the infrared spectrum of the alkaloid indicated a possibility of an ester group, a small amount of the base was hydrolyzed with alcoholic potassium hydroxide. The hydrolyzate was separated into the basic and acidic fractions. The basic fragment was a liquid, which after distillation gave analytical figures agreeing with $C_{11}H_{23}ON$. It shows also the presence of one $N-CH_3$ group. A basic monobenzoyl derivative of this compound was prepared which gave a crystalline picrolonate. The analyses of these derivatives support the formula $C_{11}H_{23}ON$ and also show that the compound contains a hydroxy group and a tertiary nitrogen.

The acidic component was extracted from the hydrolysis mixture after acidification with hydrochloric acid in a continuous ether extractor. The analysis showed that it actually was a hydrochloride of an amino acid $C_7H_7O_2N$. This amino acid gave, with a slight excess of diazomethane, an ester $C_8H_9O_2N$, m.p. 110°C. This melting point is identical with the melting point of methyl *p*-aminobenzoate and was not depressed by admixture of the ester with an authentic specimen. Also the infrared spectra of methyl *p*-aminobenzoate and our ester were identical. The amino acid had ultraviolet spectra

[1] *Manuscript received December 22, 1950.*
Contribution from the Chemistry Laboratories of the University of New Brunswick, Fredericton, N.B.

both in acidic (maxima 222 mμ, log ε = 4.08; 290 mμ, log ε = 4.25) and alkaline (maximum 266 mμ, log ε = 4.29) alcohol identical with those of *p*-aminobenzoic acid. It is therefore clear that the formula $C_{18}H_{28}O_2N_2$ is to be preferred for jaborandine and that this alkaloid is the *p*-aminobenzoic ester of amino alcohol I.

$$HO - C_{10}H_{19} = NCH_3$$
$$\text{I}$$

No further experiments have yet been done on the structure of this compound but it seems likely that it contains the nitrogen in a ring, which would probably classify it among either the piperidine or pyrrolidine alkaloids. The dehydrogenation with selenium (350 mgm.) carried out on 100 mgm. of base I, at 350°C. for eight hours in a sealed tube, gave in a 15% yield a basic product which distilled at approximately the same temperature as base I and had an absorption maximum at 263mμ, log ε 3.45. This leaves not much doubt that the dehydrogenation product is a substituted pyridine derivative and therefore base I a substituted N-methylpiperidine alcohol.

Experimental Part

Jaborandine hydrochloride

The compound was recrystallized seven times from alcohol to a constant melting point, 134°C. Sometimes the compound resolidified and melted at 154–156°C. For analysis it was dried in a high vacuum for 24 hr. at 70°C., Calc. for: $C_{18}H_{31}O_2N_2Cl$: C, 62.9; H, 9.13; N, 8.16; Cl, 10.34; N methyl, 4.37%. Calc. for: $C_{18}H_{29}O_2N_2Cl$: C, 63.35; H, 8.58; N, 8.21; Cl, 10.41; N methyl, 4.40%. Found: C, 62.61; H, 8.93; N, 8.18; Cl, 10.91; N methyl, 4.62%. *The picrolonate* was recrystallized six times to a constant melting point of 148–149°C. and dried in a high vacuum at 80°C. for 24 hr. Calc. for: $C_{28}H_{33}O_7N_6$: C, 58.9; H, 6.73; N, 14.71%. Calc. for: $C_{28}H_{36}O_7N_6$: C, 59.1; H, 6.38; N, 14.78%. Found: C, 58.97; H, 6.49; N, 14.92. The free base was liberated from the recrystallized hydrochloride and distilled in a collar flask. It boiled at 190–200°C. (outside) at 0.5 mm. Calc. for: $C_{18}H_{30}O_2N_2$: C, 70.5; H, 9.88; N, 9.14%. Calc. for: $C_{18}H_{28}O_2N_2$: C, 70.9; H, 9.29; N, 9.19%. Found: C, 70.23; H, 9.72; N, 9.42%.

Hydrolysis of Jaborandine

Jaborandine (150 mgm.) was refluxed for six hours with 8 cc. of 10% alcoholic potassium hydroxide. After this time the alcohol was taken off in a vacuum and 30 cc. of water added. The solution was extracted exhaustively with ether. The ether extract was dried and evaporated to dryness, leaving 78 mgm. of an oily base. This compound was purified by filtering through a column of 3 gm. of alumina in chloroform and fractionally distilled in a collar flask in high vacuum. It boiled sharply at 85–90°C. (outside temperature) at 2–3 mm. The middle fraction was analyzed. Calc. for: $C_{11}H_{23}ON$: C, 71.2; H, 12.52; N, 7.55; 1 active H, 0.54, N methyl, 8.1%. Found: C, 70.67; H, 12.08; N, 7.65; active H, 0.6%; N methyl, 5.26%. The base showed no ultraviolet absorption.

As the base failed to give crystalline salts and it was obviously desirable to purify it through a solid derivative, 100 mgm. was benzoylated by the usual procedure with benzoyl chloride and pyridine. A quantitative yield of a basic benzoyl compound was obtained which gave a crystalline picrolonate. The picrolonate after six recrystallizations from methanol melted sharply at 104–105°C. and was dried at 80°C. for 24 hr. in high vacuum. Calc. for: $C_{28}H_{35}O_7N_5$: C, 60.7; H, 6.38; N, 12.66%. Found: C, 60.65; H, 6.54; N, 12.83%. The free benzoylated base was liberated from the picrolonate and distilled for analysis in a collar flask. It boiled at 1 mm. at 140–150°C. (outside temperature). Calc. for: $C_{18}H_{27}O_2N$: C, 74.7; H, 9.44; N, 4.84%. Found: C, 74.61; H, 9.56; N, 5.00%. The aqueous alkaline layer after extraction of the base $C_{11}H_{23}ON$ was acidified with hydrochloric acid and extracted continuously with ether for three days. After evaporation of the ether approximately 70 mgm. of a crystalline solid remained. It was recrystallized from alcohol–ether, and after two crystallizations it melted around 200°C. with decomposition. It was shown by qualitative test to be a hydrochloride. A small sample was dried for analysis at 90°C. in high vacuum. Calc. for: $C_7H_7O_2NHCl$: C, 48.4; H, 4.61; N, 8.06%. Found: C, 47.94; H, 4.69; N, 7.98%.

The rest of the compound together with a sample obtained in a second experiment (130 mgm. in all) was dissolved in 5 cc. of methanol and just sufficient diazomethane solution was added to make the solution permanently faintly yellow. After standing for 15 min. the solution was filtered, evaporated to dryness, and the ester which formed a slowly crystallizing white oil recrystallized from a methanol–water mixture. After four crystallizations the ester had a constant melting point, 110°C., which showed no depression on admixture of the ester with a sample of methyl *p*-aminobenzoate of the same melting point. For analysis the compound was sublimed in high vacuum at 100°C. Calc. for: $C_8H_9O_2N$: C, 63.56; H, 6.00; N, 9.27; OCH_3, 20.40%. Found: C, 63.62; H, 6.08; N, 9.37; OCH_3, 20.35%. The compound gave an infrared spectrum identical with the spectrum of methyl *p*-aminobenzoate.

Acknowledgments

The author expresses his gratitude to Drs. O. Ribeiro and A. Machado, Rio de Janeiro, for their kind co-operation and a gift of crystalline jaborandine hydrochloride with which the work was performed.

This work was performed on a grant from the National Research Council, Ottawa. The analyses were done in the Microanalytic Laboratory of Dr. Rob. Dietrich in Zurich. The infrared spectra were taken partly in the National Research Laboratories, Ottawa, and partly by Messrs. Samuel P. Sadtler & Son, Inc., Philadelphia.

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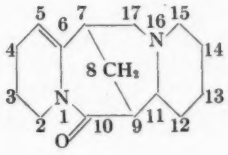
THE SYNTHESIS OF DEHYDROLUPANINES AND THE STEREOCHEMISTRY OF THE C_{15} LUPIN ALKALOIDS[1]

By Léo Marion and Nelson J. Leonard

Abstract

Two new dehydrolupanines, $C_{15}H_{22}N_2O$, have been prepared: (1) by the mercuric acetate dehydrogenation of the alkaloid *d*-lupanine, and (2) by the N-bromosuccinimide dehydrogenation of *d*-lupanine. The conversion of *d*-lupanine to its diastereoisomeric alkaloid *d*-α-isolupanine has been effected by mercuric acetate dehydrogenation followed by catalytic hydrogenation. On the basis of evidence and knowledge at present accumulated it has been possible to assign absolute stereochemical structures to a number of C_{15} lupin alkaloids.

Of the three lupin alkaloids of molecular formula $C_{15}H_{22}N_2O$ thus far characterized, namely aphyllidine (10), monspessulanine (11), and "P1" (8), the structure of only the first has been established (I). The molecular formula suggested the possible biogenetic derivation of the other $C_{15}H_{22}N_2O$ alkaloids from either lupanine (II) or hydroxylupanine (III). Accordingly, laboratory methods have been investigated for the conversion of the well known alkaloid II to compounds of composition $C_{15}H_{22}N_2O$, characterized by the formation of derivatives and hydrogenation products.



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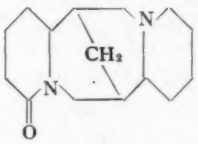

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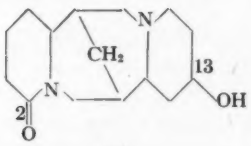

III

The position of attachment of the hydroxyl group in hydroxylupanine (III) has been ascertained as C–13[2] by Galinovsky, Pöhm, and Riedl (3) on the basis of products obtained on exhaustive chromic acid oxidation of III. Dehydration of hydroxylupanine would be expected to yield either Δ^{12}-dehydrolupanine (IV) or Δ^{13}-dehydrolupanine (V), both possible products having the unsaturation β, γ to the amino nitrogen (N–16) provided no rearrangement attended the

[1] *Manuscript received January 9, 1951.*
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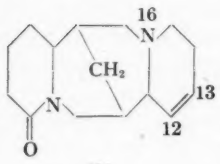
[2] *C–12 on J. F. Couch's numbering system.*

III → 

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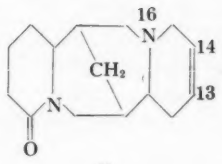
or 

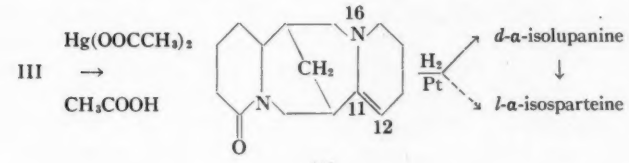
V

dehydration. Phosphorus pentoxide was employed by Galinovsky and Pöhm (2) for the dehydration of hydroxylupanine during the process of establishing the constitution of this alkaloid, but the product was not isolated. Instead, the crude intermediate was hydrogenated over palladium on charcoal directly to *d*-lupanine (II). It might be that the $\Delta^{12(13)}$-dehydrolupanine (IV or V) could be obtained more efficaciously by a milder dehydration process such as the Chugaev reaction.[3]

A new dehydrolupanine was obtained by the mercuric acetate dehydrogenation of *d*-lupanine and will be referred to as "dehydrolupanine (MA)," in the absence of an absolute determination of the double-bond position. The same reaction had been carried out by Kneuer (4), but he found that the product obtained upon loss of two atoms of hydrogen from lupanine was too unstable to be characterized. Our experience paralleled Kneuer's in that the quantity of mercurous acetate precipitated indicated the abstraction of two hydrogens from the *d*-lupanine molecule, and the "dehydrolupanine (MA)" formed was especially sensitive to air. However, we found that if the base was kept bound in the salt form by means of acetic acid, catalytic hydrogenation of the probable $C_{15}H_{22}N_2O$ intermediate could be effected before too much decomposition had taken place. When the "dehydrolupanine (MA)" in acetic acid was hydrogenated over platinum, two products were isolated in the pure state: *l*-α-isosparteine, $C_{15}H_{26}N_2$, identified as the free base and picrate, and *d*-α-isolupanine, $C_{15}H_{24}N_2O$, identified as the perchlorate.

The conversion of *d*-lupanine to these two products of the *iso* series indicates that inversion at one of the asymmetric centers of *d*-lupanine must have occurred in the dehydrogenation–rehydrogenation process. The successful conversion of *d*-lupanine to its diastereoisomer *d*-α-isolupanine is similar to the change from *l*-sparteine to *l*-α-isosparteine, effected by Winterfeld and Rauch (12) by means of mercuric acetate dehydrogenation to α-didehydrosparteine followed by catalytic hydrogenation. In both instances, the conversions must involve the same asymmetric carbon atom, and since the dehydrogenation process probably introduces unsaturation α, β to the basic nitrogen (N–16 in *d*-lupanine, both nitrogens in *l*-sparteine), it is possible to postulate the structure of the unisolated "dehydrolupanine (MA)" as Δ^{11}-dehydrolupanine (VI). The vinyl tertiary amine grouping would in part account for the instability of this

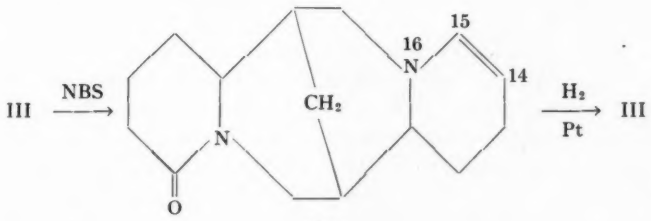
[3]*See, for example: Alexander, E. R. and Mudrak, A. J. Am. Chem. Soc. 72: 1810. 1950; Adams, R., McKenzie, S. Jr. and Loewe, S. J. Am. Chem. Soc. 70: 664. 1948; Djerassi, C. Chem. Revs. 43: 271. 1948.*



VI

dehydrogenated lupanine, in contrast to the relatively stable $\Delta^{12(13)}$-dehydrolupanine obtained in the dehydration of hydroxylupanine. The formation from VI of *l*-α-isosparteine along with the expected *d*-α-isolupanine is somewhat surprising since the catalytic hydrogenation of *d*-α-isolupanine to *l*-α-isosparteine has in the past required 2*N* hydrochloric acid solution (1), rather than glacial acetic acid, which was the solvent used in the hydrogenation of "dehydrolupanine (MA)."

Another dehydrolupanine was obtained by the dehydrogenation of *d*-lupanine by means of N-bromosuccinimide, a reagent whose application appears to be novel in the alkaloid field. The "dehydrolupanine (NBS)", $C_{15}H_{22}N_2O$, underwent decomposition when distillation of the free base was attempted, but it was readily characterized by formation of the perchlorate, m.p. 240.5–241.5°C. (dec.), $[\alpha]_D^{26}$ −125° (water). The rehydrogenation of "dehydrolupanine (NBS)" in the presence of platinum catalyst in acetic acid solution furnished *d*-lupanine in near quantitative yield, which indicated that an asymmetric center was probably not involved in the dehydrogenation–rehydrogenation process, or at least that position C-11 was not involved (as in the action of mercuric acetate). Since sparteine, with no amide linkage, undergoes reaction with N-bromosuccinimide (5) and since the reagent probably introduces unsaturation α, β to the basic nitrogen (N–16) in *d*-lupanine, the structure of the "dehydrolupanine (NBS)" may be represented tentatively as Δ^{14}-dehydrolupanine (VII).



VII

The ease of dehydrogenation of *d*-lupanine with N-bromosuccinimide was somewhat surprising. It was necessary only to mix the two reactants in chloroform solution, and the product was worked up immediately. Neither peroxide nor heat nor an extended reaction time had any beneficial effect on the dehydrogenation process. The action of N-bromosuccinimide in the dehydrogenation of amines is being investigated further.

On the basis of evidence and knowledge at present accumulated, it should be possible to assign absolute stereochemical structures to the members of the C_{15} family of lupin alkaloids. With the alkaloid thermopsine identified by Cockburn and Marion (1) as structurally similar but stereochemically different from anagyrine, the two racemates of $C_{15}H_{20}N_2O$ (ring A = α-pyridone) have been satisfactorily characterized as *dl*-anagyrine and *dl*-thermopsine. *d*-Anagyrine is the only one of the four optically active forms which has not yet been found in nature. Considering only one of the enantiomorphs of each racemate, the configuration of the diastereoisomers can be represented by formula VIII in which the relation of the 7, 9-methylene bridge to the C_{11}-hydrogen is *cis* and by that in which the relation is *trans* (IX) (see Fig. 1). The $C_{15}H_{24}N_2O$ compounds (ring A = α-piperidone) can exist in four racemic forms due to the *different* asymmetric carbons C_6 and C_{11} and the $C_{7,9}$-asymmetric center. With only one enantiomorph of each racemic pair indicated in Fig. 1, the relations between the C_6-hydrogen, the $C_{7,9}$-methylene bridge, and the C_{11}-hydrogen in these four diastereoisomeric forms are indicated as follows: *cis-cis* (X), *trans-cis* (XI), *cis-trans* (XII), and *trans-trans* (XIII). One of the racemates corresponds to *dl*-lupanine, of which both forms have been found in nature, and a second racemate corresponds to *dl*-α-isolupanine (*dl*-tetrahydrothermopsine), of which only the *d*-form has thus far been found in nature (9). It is as yet unknown whether the $C_{15}H_{24}N_2O$ alkaloidal products: nonalupine, sophocarpine, and dihydromonspessulanine have the same basic ring structure as lupanine; therefore, the distribution of the other two racemates structurally related to lupanine and α-isolupanine remains in doubt at present. The $C_{15}H_{26}N_2$ compounds, by contrast, have two *similar* asymmetric carbons, C_6 and C_{11}, and the asymmetric center at $C_{7,9}$. Three racemates exist, and the relations between the asymmetric centers in a representative of each, as indicated in Fig. 1, are as follows: *cis-cis* (XIV), *cis-trans* (XV), and *trans-trans* (XVI). Both *d*- and *l*-sparteine have been found in nature, and *l*-α-isosparteine has recently been isolated from natural sources for the first time by Marion, Turcotte and Ouellet (9). "β-Isosparteine" has not yet been characterized.

A provisional stereochemical structure has been assigned to sparteine (6) on the basis of accumulated evidence as to the structural similarity but configurational difference between rings B and C in sparteine and its derivatives. The stereochemical structure is represented by the *cis-trans* form (XV) in Fig. 1. If this structure is correct, then the lupanine and anagyrine progenitors of sparteine must be, respectively, either the *trans-cis* $C_{15}H_{24}N_2O$ (XI) and the blank-*cis* $C_{15}H_{20}N_2O$ (VIII), *or* the *cis-trans* $C_{15}H_{24}N_2O$ (XII) and the blank-*trans* $C_{15}H_{20}N_2O$ (IX). The key to the configuration of the progenitors of sparteine lies in the stereochemical course of the hydrogenation reaction which converts anagyrine to lupanine; i.e., whether the hydrogen becomes attached to the 6-carbon of anagyrine *cis* to the $C_{7,9}$-methylene bridge or *trans* to it. A careful inspection of scale molecular models (Fisher–Hirschfelder–Taylor and Stuart) indicates that there is a real difference between the two faces of the $C_{15}H_{20}N_2O$ molecule in ease of approach toward hydrogen on a catalyst surface.

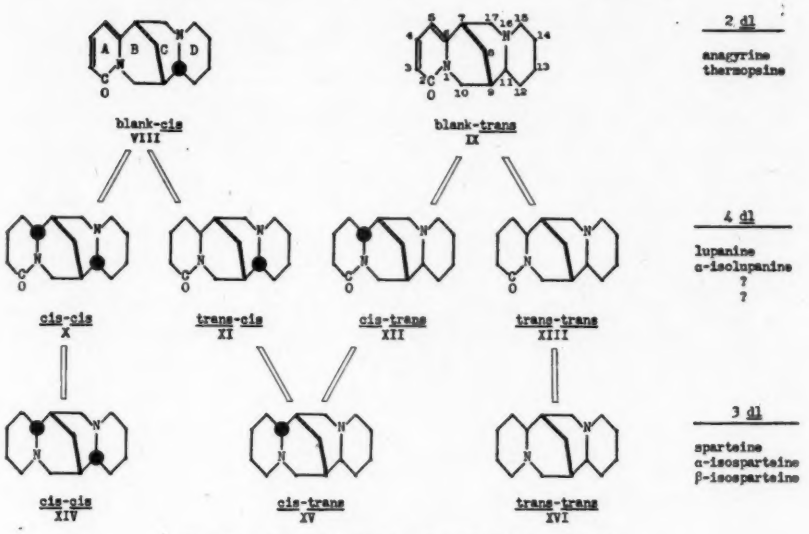


FIG. 1. *Stereochemical family—C_{15} lupin alkaloids.*

Approach at C_6 *trans* to the methylene bridge is appreciably masked by the atoms of rings B, C, and D, whereas approach at C_6 *cis* to the $C_{7,9}$-methylene bridge is relatively unhindered. On the basis of this model inspection and the recognized surface nature of the catalytic hydrogenation process (7), the assumption can therefore be made that hydrogen enters the anagyrine molecule *cis* to the $C_{7,9}$-methylene bridge in the formation of lupanine. This limits the structure of lupanine to either the *cis-cis* or the *cis-trans* $C_{15}H_{24}N_2O$ type, and a decision can be made in favor of the latter (XII), since sparteine, which is obtained from lupanine, has the *cis-trans* $C_{15}H_{26}N_2$ structure (XV) and only the *cis-trans* $C_{15}H_{24}N_2O$ type can be its precursor. Thus, it becomes possible to assign stereochemical structures to the entire sparteine family. Lupanine (both *d* and *l*) has the *cis-trans* configuration (XII), and its precursor anagyrine (both *d* and *l*) can only have the blank-*trans* configuration (IX). On the basis of this sole assumption that hydrogen enters the $C_{15}H_{20}N_2O$ molecule at C_6 *cis* to the methylene bridge, it also becomes possible to assign stereochemical structures to thermopsine, α-isolupanine (tetrahydrothermopsine), and α-isosparteine. (An inspection of the scale models shows that *cis* approach to the methylene bridge is easier than *trans* no matter whether the C_{11}-hydrogen is *cis* or *trans*.) Thermopsine has been shown to be convertible to α-isolupanine (tetrahydrothermopsine) by hydrogenation over platinum in acetic acid, and α-isolupanine, to α-isosparteine by hydrogenation over platinum in hydrochloric acid (1, 9). Thermopsine must have the blank-*cis* $C_{15}H_{20}N_2O$ structure (VIII) since it is diastereoisomeric with anagyrine; *cis*-hydrogenation at C_6 would produce the *cis-cis* $C_{15}H_{24}N_2O$ structure (X), which is therefore α-isolupanine. These blank-*cis* (VIII) and *cis-cis* (X) progenitors require that*

α-isosparteine, $C_{15}H_{26}N_2$, have the structure *cis-cis* (XIV). It will be noted that there is thus stereochemical consistency in the two families: anagyrine → lupanine → sparteine; thermopsine → α-isolupanine → α-isosparteine.

It was shown earlier that sparteine can be converted to isosparteine by mercuric acetate dehydrogenation followed by catalytic hydrogenation (12), and in the present work, the conversion of lupanine to α-isolupanine by a similar process has been described. The conversion of lupanine, with the *cis-trans* structure (XII), to α-isolupanine, with the *cis-cis* structure (X), necessitates the introduction of a double bond at C_{11}. If the subsequent hydrogenation (over platinum in acetic acid) is to produce the *cis-cis* $C_{15}H_{24}N_2O$ molecule, the hydrogen must enter *cis* to the $C_{7,9}$-methylene bridge. This is entirely consistent with the assumption as to stereospecificity of hydrogenation reached earlier on the basis of the scale models. The dehydrogenation–rehydrogenation of sparteine (XV) must proceed in an analogous manner: introduction of a double bond at C_{11}, followed by catalytic addition of hydrogen at C_{11} *cis* to the $C_{7,9}$-methylene bridge to produce the *cis-cis* α-isosparteine (XIV).

Experimental*

Dehydrogenation of d-Lupanine by N-Bromosuccinimide. "Dehydrolupanine (NBS)"

A solution of 1.812 gm. (5.2 millimoles) of *d*-lupanine perchlorate in water was treated with concentrated ammonium hydroxide, and the basic solution was extracted with chloroform. The combined chloroform extracts were evaporated to dryness, and the residue was redissolved in anhydrous chloroform. To this solution was added 1.07 gm. (6.0 millimoles) of N-bromosuccinimide in 125 ml. of anhydrous chloroform. The resulting yellow solution was shaken briefly (two to three minutes), then extracted several times with aqueous sodium hydroxide. The chloroform layer was washed with water, and the chloroform was removed by distillation, finally *in vacuo*. The residue was dissolved in methanol, and to the methanolic solution was added 72% perchloric acid until the solution was just acid to Congo red. An immediate separation of colorless triangular plates resulted, and a total of 1.333 gm. (74%) of *perchlorate* was collected. After two recrystallizations from methanol containing a trace of ether the perchlorate melted at 240.5–241.5°C., with decomposition; $[\alpha]_D^{26}$ −125° ± 1° (c = 1.10, water). Found: C, 51.65; H, 7.22; N, 8.03%. Calc. for $C_{15}H_{22}N_2O \cdot HClO_4$: C, 51.94; H, 6.68; N, 8.08%.

Hydrogenation of "Dehydrolupanine (NBS)"

When an attempt was made to distil the free base, obtained by basification with aqueous sodium hydroxide followed by chloroform extraction and evaporation of the chloroform, no distillate was obtained at 0.2 mm. up to 230°C. and intensive resinification occurred. For catalytic hydrogenation, 300 mgm.

* *All melting points are corrected.*

(1.2 millimoles) of "dehydrolupanine (NBS)" was used, as obtained from a solution of the perchlorate by basification and chloroform extraction followed by evaporation of the chloroform. A solution in 10 ml. of glacial acetic acid did not absorb hydrogen when palladium on charcoal was used as the catalyst, but absorbed one molar equivalent of hydrogen in two hours when platinum oxide catalyst was used. The solution was filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was treated with 5% aqueous sodium hydroxide and extracted with chloroform. The chloroform extracts were washed with water and evaporated to dryness. The residue was distilled at 0.15 mm. and a bath temperature of 135–145°C. The distillate was converted directly to the perchlorate by adding perchloric acid to a methanol solution; m.p. 210–211°C., undepressed on mixture with an authentic sample of *d-lupanine perchlorate* (m.p. 210–211°C.). The specific rotation was also identical with that of *d*-lupanine perchlorate, as determined in aqueous solution.

Attempted Further Dehydrogenation of "Dehydrolupanine (NBS)"

A solution of "dehydrolupanine (NBS)" in chloroform, made by decomposition of 1.20 gm. (3.46 millimoles) of "dehydrolupanine (NBS)" perchlorate, was treated with a solution of 0.678 gm. (3.81 millimoles) of N-bromosuccinimide in 100 ml. of chloroform. The resulting solution changed from colorless to yellow to orange, then back to straw yellow, just on shaking a few minutes. The chloroform solution was extracted three times with aqueous sodium hydroxide, washed with water, and evaporated to dryness. The product was not homogeneous, as evidenced by the perchlorate which separated as a mixture of reddish oil and crystals that resisted purification.

Dehydrogenation of d-Lupanine by Mercuric Acetate. "Dehydrolupanine (MA)"

A solution of *d*-lupanine in chloroform obtained by decomposition of 1.406 gm. (4.03 millimoles) of *d*-lupanine perchlorate, was evaporated to dryness, and the residue was redissolved in 50 ml. of 5% acetic acid in water. To this acetic acid solution was added 10.3 gm. (32.2 millimoles) of mercuric acetate, and the resulting solution was stirred under a nitrogen atmosphere on the steam bath for five hours. The mixture was cooled and filtered, and 1.9 gm. (7.3 millimoles) of mercurous acetate was collected. This corresponds to the removal of two hydrogen atoms from lupanine. The filtrate was saturated with hydrogen sulphide and the insoluble mercuric sulphide was removed by filtration. The final filtrate was evaporated to near dryness *in vacuo*, a small amount of 10% sulphuric acid was added to dissolve the residue, and excess aqueous sodium hydroxide and chloroform were added. The aqueous layer was rapidly extracted with further portions of chloroform, the combined chloroform extracts were washed with water, and glacial acetic acid was added to the chloroform solution. If the precaution of adding acetic acid was not followed, decomposition of the base in chloroform solution occurred very rapidly. It was not possible to isolate the "dehydrolupanine (MA)", and attempts to form perchlorate or picrate derivatives were not successful.

Hydrogenation of "Dehydrolupanine (MA)"

The chloroform – acetic acid solution was evaporated to dryness *in vacuo*, and the residue was redissolved in 35 ml. of glacial acetic acid. Hydrogenated at room temperature and atmospheric pressure over 300 mgm. of platinum oxide catalyst, the material (assuming it to be $C_{15}H_{22}N_2O$) absorbed one mole equivalent of hydrogen. The catalyst was removed by filtration and the acetic acid, by evaporation *in vacuo*. The residue was treated with 5% aqueous sodium hydroxide and chloroform, and the aqueous layer was further extracted with chloroform. The combined extracts were washed with water and evaporated to dryness *in vacuo*. The residue was distilled *in vacuo* and two fractions were collected: 66.1 mgm. of colorless needle crystals which sublimed at 0.07 mm. and a bath temperature of 70–80°C.; 140.5 mgm. of colorless oil which distilled at 0.08 mm. and a bath temperature of 125–140°C. The solid fraction was resublimed for analysis. Found: C, 75.62; H, 11.28%. Calc. for $C_{15}H_{26}N_2$: C, 76.86; H, 11.18%. For $C_{15}H_{26}N_2 \cdot H_2O$: C, 71.38; H, 11.18%.

The analysis was suggestive of *l-α-isosparteine* partially hydrated, as was the melting point behavior (melting at 104°C., clearing at 115°C.). When mixed with a sample of freshly sublimed *l-α*-isosparteine, the melting point behavior was identical. A *picrate* of the solid fraction was made in ethanol and recrystallized from methanol; m.p. 221°C., with decomposition, undepressed on mixture with an authentic sample of *l-α*-isosparteine picrate (m.p. 221°C., with decomposition).

The second or liquid fraction (see above) was converted to the perchlorate in methanol solution. A fractional crystallization of the crude salt which first separated gave a more soluble perchlorate, recrystallized from methanol–ether, which melted with decomposition at 248–249°C. This perchlorate was not depressed in melting point when mixed with the perchlorate of an authentic sample of *d-α-isolupanine* (m.p. 248–249°C. with decomposition). The less-soluble perchlorate fractions appeared to be mixtures, indicating the presence but not the identity of a second constituent of the high boiling fraction resulting from dehydrogenation–hydrogenation.

Acknowledgment

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THE SEPARATION OF THE RARE EARTHS BY ION-EXCHANGE PROCEDURES[1]

By F. T. Fitch and D. S. Russell

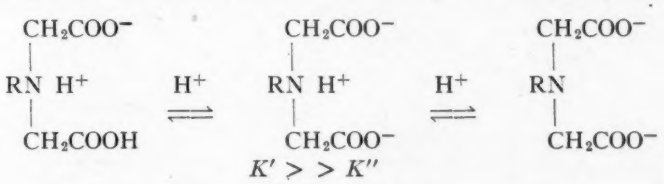
Abstract

The iminodiacetic acids have been examined as possible complexing agents in the chromatographic separation of the rare earths with cation exchange resins. The ammonium salts of these acids form complex ions involving both one and two iminodiacetate ions of increasing stability with the rare earth elements of greater atomic number. Methods greatly reducing the time and material requirements are presented for the separation of the cerium group elements with nitrilotriacetic acid.

Introduction

Considerable success in the separation of the rare earth elements has been achieved recently in the application of chromatographic techniques to cation exchange resins (6, 18, 21). Elution with a complexing agent, generally citric acid, was found to move the adsorbed rare earth ions through an exchanger bed at different rates. The elements of higher atomic number, which formed the more stable complex ions with citric acid, were collected in correspondingly earlier portions of the eluate. Careful control of pH and ion concentrations was necessary to achieve the desired ion-exchange and exploit to a maximum the slight differences in behavior of the elements (19, 20). However, with the large volumes of eluate and further separations required, the purification of appreciable quantities of these elements still remained laborious.

The iminodiacetic acids, $RN(CH_2COOH)_2$, are excellent complexing agents for most di- and trivalent cations, and interest in the present work has centered on their development as eluting agents for the rare earth elements. These compounds are weak acids with "zwitterion" or dual type ion formation causing considerable difference in the dissociation of the acid groups.



When partially neutralized, these acids readily form complex ions with most cations, chelate formation occurring through the nitrogen and acid groups. In many cases, basic ions are indicated at high pH values and often the metal hydroxide may be precipitated on addition of strong base.

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Hydrogen ion is liberated from the zwitterion in chelate formation. Titration of the acids with base in the presence of various cations gives some indication of the extent of complex ion formation at different pH values and may permit an estimation of both the acid dissociation and complex ion instability constants. With nitrilotriacetic acid, Schwarzenbach and Biedermann (14) have reported that a mixture of both the mono- and di-acid complex ions was formed by lanthanum or cerous ions with some indication of greater stability for the complexes of the latter element. Beck (2) has found in fractional precipitation experiments additional evidence for the greater stability of the complex ions of nitrilotriacetate with the rare earths of higher atomic number and also confirmed the existence of the di-acid complex ion, $M(N(CH_2COO)_3)_2^{---}$, by precipitation with $Co(NH_3)_6^{+++}$.

At pH 5 to 7, in which range the elution of the rare earths was found to occur, the iminodiacetic acids are present almost entirely as either the HA^- or HA'^{--} anion. Titration experiments have indicated that the complex rare earth ions would be MA^+ and largely MA_2^-, where M represents the trivalent cation. The principal equilibria involved in the complexing and exchange reactions between the eluting solution and the ammonium form of the resin may be written as follows:

$$HA^- \overset{K''}{\rightleftharpoons} H^+ + A^=,$$

$$MA^+ \overset{K_c'}{\rightleftharpoons} M^{+++} + A^=,$$

$$MA_2^- \overset{K_c''}{\rightleftharpoons} MA^+ + A^=,$$

$$\text{and } M^{+++} + 3NH_4R \overset{K_{ex}}{\rightleftharpoons} 3NH_4^+ + MR_3,$$

where R represents the anionic species of the resin. Applying the mass law to these equilibria, an expression similar to that applied to the citric acid system (7) may be obtained for the rare earth distribution coefficient, K_d, as follows:

$$K_d = \frac{(MR_3)}{(MA^+) + (MA_2^-)}$$

$$= \left(\frac{1}{1 + \dfrac{K''(HA^-)}{K_c''(H^+)}}\right) \frac{K_{ex}K_c'}{K''} \frac{(NH_4R)^3}{(NH_4^+)^3} \frac{(H^+)}{(HA^-)}$$

With the rare earth present to a large extent as the di-acid complex ion, MA_2^-, under the elution conditions, the distribution coefficient would tend to approach the following expression:

$$K_d = \frac{K_{ex}K_c'K_c''}{(K'')^2} \frac{(NH_4R)^3}{(NH_4^+)^3} \frac{(H^+)^2}{(HA^-)^2}$$

Thus, an increase in pH, iminodiacetic acid or ammonium ion concentration could be expected to increase the proportion of rare earth in solution and quicken the elution of the cation from the exchanger. The displacement of ammonium ion from the resin by appreciable quantities of a foreign cation or rare earth ion should decrease the distribution coefficient. It has been found convenient to use a separation factor (7, 9), namely, the ratio of the respective distribution coefficients, in estimating the possibility of separating a pair of cations. The data available are insufficient to permit prediction of the separation factors for the rare earth elements but the difference in effect of neodymium and lanthanum on the titration curves was great enough to indicate convenient separations throughout the group. The use of the iminodiacetic acids as eluting agents has been investigated and, in particular, procedures employing nitrilotriacetic acid have been developed for the separation and purification of the cerium group elements.

Experimental

Materials

Resin.—Amberlite I.R. 120, 20–35 mesh, Resinous Products and Chemical Company.

Rare earth.—Crude didymium oxide, Lindsay Light and Chemical Co., and resulting column preparations.

Iminodiacetic acids.—A number of these acids were synthesized by heating solutions of the corresponding amino acid (or hydrazine) and chloroacetic acid with controlled addition of the calculated quantities of potassium hydroxide or carbonate to remove the resulting hydrochloric acid. The products were recovered either as the insoluble acid or metal salt as described in the literature. This general procedure was followed in the preparation of nitrilotriacetic acid (10), hydrazinodiacetic acid (1), benzyl iminodiacetic acid (3), and *o*-carboxyaniline-N-N-diacetic acid (17). In the preparation of nitrilotriacetic acid, yields of about 80% theoretical were obtained using chloroacetic instead of iodoacetic acid, but often larger quantities of hydrochloric acid than those indicated were necessary for the precipitation of the product.

Rare Earth Analysis

Total rare earth was determined gravimetrically by precipitation with oxalic acid and ignition to the oxide. Careful control of precipitation conditions and reagent quantities was necessary, particularly with lanthanum, to avoid small filtrate losses apparently due to a slight solubility of the precipitate (8, 13). The rare earth was precipitated from solution, heated just to boiling, by the addition of 10% oxalic acid (4 ml. per 100 ml. sample) and then allowed to stand for two hours on a steam bath. After cooling rapidly to 0°C. in a salt–ice bath with frequent stirring, the precipitate was filtered from solution and ignited at 800°C. for weighing. These precipitation conditions with a final cooling in tap water were followed roughly in the recovery of rare earth from

the large volumes of column eluate, and material losses of only 2–3% were encountered throughout the extensive handling of a column run.

Samarium, neodymium, and praseodymium were determined by their absorption spectra as described by Rodden (12) and Moeller and Brantley (11), employing a Cary Recording Spectrophotometer, Model 11M. Special cells with a light path of 2 cm. were designed to handle 2–3 ml. solutions of the oxide samples dissolved in 2% hydrochloric acid. For this determination absorption bands were employed at the following wave lengths: Pr, 444.5 mμ; Nd, 521 mμ; Sm, 402 mμ. Oxides, that had been purified extensively by column elution, were used in the preparation of samples and synthetic mixtures to obtain standard curves. As the examination of the absorption spectra of these materials gave no suggestion of contamination, the purity was better than 99.5% for Pr and Nd and 99% for Sm. Only at high concentrations did the absorption bands give any indication of deviation from Beer's law and these elements appeared to have negligible effect on the absorption spectra of one another. In mixed oxides samples of 200 mgm., these elements could be determined down to approximately 1% Pr, 2% Nd, and 3% Sm and detected at appreciably lower concentrations.

Titration of the Iminodiacetic Acids

The iminodiacetic acids may be titrated to a sharp end point prior to neutralization of the last acid group (16, 17). The additional acid liberated owing to complex formation was determined throughout the pH range of this end point by titration of the various acids with sodium hydroxide (0.1 and 0.5 *N*) in the presence of neodymium and lanthanum chloride. A Beckman pH meter, model G, was used in the titrations, and special care was required to reach equilibrium values in the initial stages owing to the relative insolubility of many of these acids.

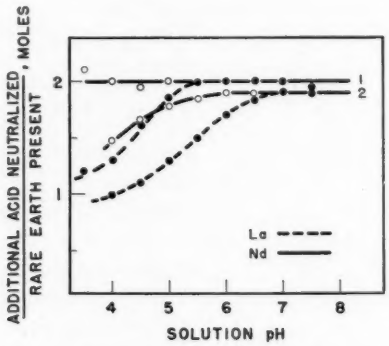

FIG. 1. *Nitrilotriacetic acid neutralized per mole rare earth due to complex formation. Rare earth, 0.33 millimole; initial volume, 100 ml. Nitrilotriacetic acid: 1. 5.0 millimole 2. 1.0 millimole.*

The acid liberated through complex formation has been shown for several cations to approach a definite mole ratio, acid/cation, indicative of the ion formed (15). Both neodymium and lanthanum, as representative of the cerium group, were found to form a complex ion under optimum conditions involving two nitrilotriacetate groups as shown in Fig. 1. At lower pH values and concentrations, incomplete reaction led to a mixture of the mono- and di-acid complex ions. Under these conditions, neodymium tended to react more completely, indicative of complex ions of greater stability for the rare earth elements of higher atomic numbers.

In general, the reactions of the various iminodiacetic acids resembled those of nitrilotriacetic acid, indicating both a greater stability for the complexes of neodymium and the formation of a di-acid complex ion under optimum conditions. However, the iminodiacetic acids possessing the greater number of acid groups of possible use in complex formation were found to form ions of greater stability as suggested in Fig. 2. These titration curves were helpful in evaluating the probable elution characteristics of the various acids, indicating the extent of complex formation and the differences in behavior of the rare earths. For example, column experiments readily confirmed the advantages of using nitrilotriacetic acid (Fig. 1) as an eluting agent at low pH's.

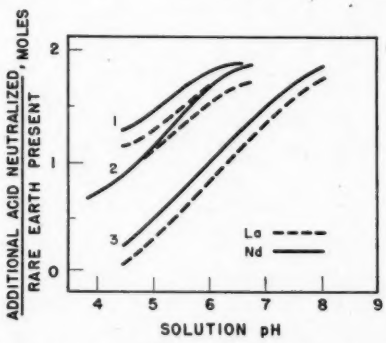

FIG. 2. *Acid neutralized per mole rare earth due to complex formation. Rare earth, 0.33 millimole; initial volume, 100 ml. 1.0 millimole acid: 1. o-Carboxy aniline-N-N-diacetic acid 2. Hydrazinodiacetic acid 3. Benzyl iminodiacetic acid.*

Elution Procedures

A column bed was prepared on a base of sand similar to those used with citric acid (5) and connected to the sampling apparatus as shown in Fig. 3. The resin was conditioned before use by treating it several times alternately with hydrochloric acid 1:3 and ammonium chloride 15%. A long illuminated tube fitted with a small spectroscope permitted the identification of the elements in the eluate by their characteristic absorption spectra as they were removed from the column.

Continuous sampling during unattended operation was accomplished by allowing the eluate to overflow at different levels from the delivery tube into closed liter Erlenmeyer flasks with standing tubes to force delivery into the following receiver. The small losses of mixed eluate and the slight contamination from level adjustments were negligible compared to the total sample volumes. With small volumes of eluate, sampling was accomplished by an intermittent siphoning pipette with an extended delivery arm. An escape mechanism actuated by the weight of each sample caused the pipette to deliver into consecutive receivers.

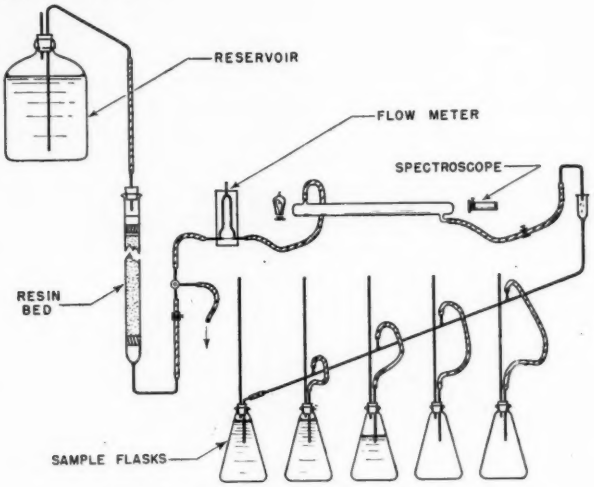

FIG. 3. *Apparatus for column separations.*

All of the various iminodiacetic acids were found to elute the rare earths from the resin bed with varying degrees of separation. The best results were obtained with complexing agents capable of forming complex ions of appreciably different strengths with the various earth elements and of a suitable stability to produce the desired exchange on passage through the column. Desirable elution characteristics and a convenient preparation in large quantities made nitrilotriacetic acid most suitable for general use and, thus, the following description is concerned mainly with this acid.

The rare earth was adsorbed on the resin (ammonium form) from a chloride solution of approximately pH 2. A nitrilotriacetic acid solution, partially neutralized with ammonium hydroxide, was passed slowly through the resin bed to elute the earth elements in order of decreasing atomic number. Allowance was made for the slower movement of the latter elements, as the elution progressed, by increasing the strength of the eluate, generally with the addition of ammonium acetate buffer. An initial treatment with eluate at low concentrations served both to remove the traces of the head elements and to band more clearly on the adsorbent the major constituents which then were removed

with excellent separations at increased concentrations. Careful control of conditions in this manner resulted in a sharp separation, on the resin, of the last element in the sample, generally lanthanum, which was recovered rapidly with stripping solution, a buffered concentrated elutriant for the quick removal of cations.

In treating a sample from 25 gm. of crude didymium oxide, the rare earth was adsorbed from a liter of the chloride solution, pH 2, on amberlite I.R. 120 resin, to form the upper 32 cm. of a resin bed, 3.2 by 160 cm. After an additional water wash of several liters, a 0.5% nitrilotriacetic acid solution at pH 5.0 was passed through the column at a rate of approximately 40 to 50 ml. per sq. cm. per hr. to remove the traces of head elements and most of the samarium. Then the eluate was buffered with 0.25% ammonium acetate at the same pH to recover neodymium, praseodymium, and cerium consecutively at much higher concentrations. At this point lanthanum remained adsorbed on the resin bed, and prolonged elution was avoided by removing the lanthanum rapidly with a stripping solution of 1% nitrilotriacetic acid and 4% ammonium chloride at pH 7 to 8. In this way, the separation of this mixture was accomplished with only 40 to 50 liters of eluate, as shown in Fig. 4. The resin bed was treated with hydrochloric acid (1:3) and then 15% ammonium chloride solution before further use.

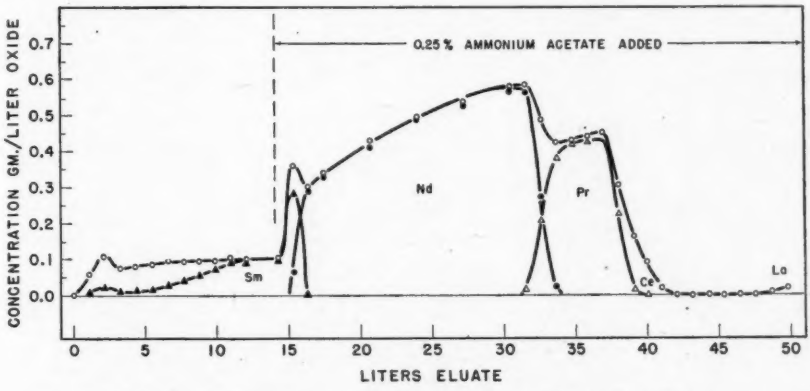

FIG. 4. *Elution curve of crude didymium with nitrilotriacetic acid.*

Although the small quantities of samarium and head elements were separated rather indifferently during the initial elution at low concentrations, the advantage of this treatment was demonstrated in the later separations of the major constituents. These elements overlapped to only a minor extent, with lanthanum actually remaining adsorbed on the resin bed during the passage of several liters of eluate. In the greater part of these elements collected, the amounts of impurities present were below detectable limits. Certain features of the elution curve reflected the operating technique, particularly the peak due to the rapid removal of samarium with the change in elution conditions.

The scale of these procedures could readily be reduced with minor modifications in concentrations, flow rate, and pH. Columns 1 by 50 cm., separating 3 gm. didymium oxide gave elution concentrations of 3 mg. per ml. at a flow rate of 10 cm. per sq. cm. per hour. Long narrow resin beds, i.e., 0.6 by 70 cm., and smaller samples, 100 to 250 mgm., with unbuffered nitrilotriacetic acid solution were used to accentuate the separations sufficiently to produce a pronounced break in the elution between lanthanum and the group elements. With another acid, hydrazinodiacetic acid, this characteristic was pronounced enough to be of possible analytical use in the separation of lanthanum (4).

Discussion

More effective separations were obtained at pH values of nearly 5 with nitrilotriacetic acid, a range in which the titration curves had indicated an appreciable difference to exist between lanthanum and neodymium in the extent of complex formation. A decrease in pH occurred in the eluate on passage through the bed which appeared to be indicative largely of the resulting complex formation and movement of the earth. Under set operating conditions, only slight variations in output pH were noted between the various elements, of little help in controlling operations. Since these solutions have negligible buffering action in this range, the rate of elution appeared to be somewhat self-regulating with the extent of complex formation rather than the input pH being the controlling factor so that little difference was noted between pH 5 to 7. With ammonium acetate added as buffer, the rate of elution became more dependent upon the input pH, and the more uniform exchange conditions existing throughout the resin bed appeared to be an additional advantage. With this buffer a higher elution rate was found which was attributed largely to the increased ammonium ion concentration. Although difficult to assess, flow rate did not appear to be a critical factor in the ranges indicated.

The elements of higher atomic number were removed earlier and at higher concentrations from the resin bed. Elution of the earths began almost immediately and generally was continuous through the series, with eluate concentrations varying for individual quantities and relative sample size. Little cross-contamination occurred between the concentration bands and, thus, the quantities of preceding material influenced the point at which any element was eluted from the resin bed. A few cations were found to act as separating elements between these concentration bands but their use was not developed fully. Of most interest with nitrilotriacetic acid was the effect of cadmium in spreading the samarium and neodymium bands further apart.

The effect of the column dimensions was roughly the same as in the case of citric acid (19, 20). Little further separation was gained by increasing the length of a column beyond a definite limit, although with a fixed volume of resin the characteristics of the elution curves became more pronounced as the ratio of length to diameter was increased.

Raising the elutriant above room temperature appeared to increase the exchange occurring in the resin bed, leading to a more definite banding of the elements and requiring greater eluate volumes. This effect was sufficiently marked at 50°C. to yield comparable results from resin beds, one third to one fifth the usual volume, in smaller volumes of more concentrated eluate.

Although not examined in detail, the column action showed appreciable deviation from the ideal picture of well defined concentration bands displaced in a regular manner through the adsorbent. Sectional analysis indicated a tendency to marked banding in a region of high concentration on the upper section of the column but with low diminishing concentrations extending downward through the greater part of the resin bed. The elution action appeared to combine this initial banding operation with the rapid washing of the most soluble ion through the remainder of the bed, limited exchange in this region leading to some further purification. As the bulk of the more soluble elements was removed some approach to a more normal banding and displacement action was indicated.

Acknowledgment

The authors wish to express their gratitude to Dr. R. N. Jones and Mr. D. S. Keir of the National Research Laboratories, for their aid in conducting the spectrophotometric analysis of the rare earths. The assistance of Mr. W. G. Humphreys in carrying out some of the operations is gratefully acknowledged.

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EXCHANGE REACTIONS OF ALICYCLIC IODIDES WITH SODIUM IODIDE[1]

By Sylvia F. Van Straten, R. V. V. Nicholls, and C. A. Winkler

Abstract

The activation energies for the exchange reactions between sodium iodide and cyclopentyl, cycloheptyl, cyclooctyl, cyclopentadecyl, and 2-heptyl iodides were all in the range 21 ± 1 kcal. per mole. For cyclohexyl iodide the value was 25.6 kcal. per mole. This difference seems to be explained from a consideration of the initial potential energies of the various rings. An inverse relation appears to exist between log PZ and $\frac{1}{N}$, the reciprocal of the number of carbon atoms in the iodides investigated, for all the iodides except cyclopentyl iodide. This might be due to a change in the number of equivalent constellations in the activated state.

Introduction

Several studies with substituted alicyclic compounds (4-6, 8-11), in particular those involving nucleophilic substitution reactions of alicyclic halides (2, 12), have demonstrated an apparently anomalous behavior of cyclohexyl derivatives. With a view to obtaining more information about such systems, the type of reaction involved has been made as simple as possible in the present investigation, by studying the exchange reaction between iodide ion and alicyclic iodides. The exchange of iodide ion with 2-heptyl iodide was also studied for purposes of comparison, since exchange occurs at a secondary carbon atom in this compound, as in alicyclic iodides.

Experimental and Results

Radioactive iodine, I^{131}, was obtained from the National Research Council, Atomic Energy Project, Chalk River, Ontario, as carrier-free sodium iodide.

Sodium iodide (Merck Reagent grade) dried for several days at 110°C. was used as diluent.

Absolute ethanol was used as solvent.

The alicyclic iodides were all prepared by treating the corresponding alcohol with 57% hydriodic acid.

Cyclopentanol, cyclohexanol, and 2-heptanol were obtained commercially (Eastman Kodak). Cycloheptanol was prepared by cyclizing suberic acid (City Chemical Company, New York) to cycloheptanone (13), followed by reduction of the ketone with aluminum isopropoxide and isopropyl alcohol. Cyclooctanol and cyclopentadecanol were also obtained in a similar manner by reduction of the corresponding ketones.*

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* *We are indebted to the University Chemical Laboratory, Cambridge, England, and to Firmenich and Company, Geneva, Switzerland, for gifts of the cyclooctanone and cyclopentadecanone ("exaltone") respectively.*

The exchange reactions were followed by withdrawing samples from appropriate thermostats at suitable time intervals, and separating the organic and inorganic iodides by benzene–water extraction. Silver iodide was precipitated from each fraction by the addition of alcoholic silver nitrate. The precipitate was washed with methanol to remove benzene, heated on a steam bath with concentrated nitric acid until brown fumes appeared, washed again twice with water and twice with methanol, and slurried in dry methanol. A sample of the slurry, contained within a glass ring, was evaporated on a glass plate and the rate of exchange determined from the change in specific activity, using standard counting equipment.

Two series of experiments were made with each alicyclic iodide, except cycloöctyl iodide, which was available in amount sufficient only for one limited series of experiments. The order of the reaction was determined for each compound by varying the initial concentrations, and the activation energy determined for a range of temperature of 20°–30°C.

The reactions were all found to conform satisfactorily to second order behavior. There was some discrepancy, for which no satisfactory explanation can be given, between the values of the second order rate constants at a given temperature in the two series of reactions with a given organic iodide. However, the activation energies obtained for the two series agreed within 400 cal. per mole, except with cyclopentyl iodide where the two values differed by 800 cal. per mole. The activation energy lines for one series of experiments with each compound (except cycloöctyl iodide) are shown in Fig. 1.

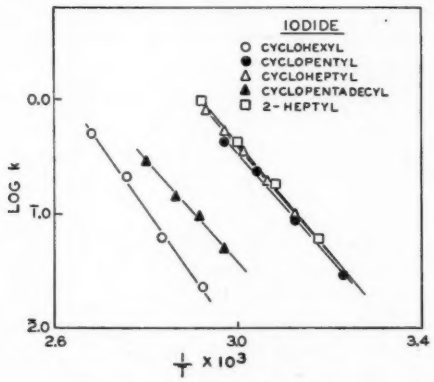

FIG. 1. *Typical Arrhenius lines.*

The data given in Tables I and II are typical of the results obtained in the study as a whole. For calculation of activation energies, the value of k used at each temperature was the average of the individual values, usually at least four, for different reaction times.

In Table III are summarized the results for the various reactions studied.

TABLE I

THE EXCHANGE OF CYCLOHEXYL IODIDE WITH SODIUM IODIDE
Concentration: RI, 0.30 *M.*; NaI, 0.30 *M*

Temp., °C.	Reaction time, min.	% Exchange	k(cc.$-$mole$^{-1}-$sec.$^{-1}$) $\times 10^2$
69.0	198	7.3	0.023
	258	7.8	0.024
	318	11.9	0.024
	383	13.5	0.022
79.9	180	17.8	0.065
	181	17.1	0.062
	240	22.2	0.063
	300	25.2	0.060
	360	30.0	0.060
89.8	120	32.0	0.195
	150	40.2	0.208
	190	50.0	0.223
	240	58.2	0.222
100.0	30	24.5	0.576
	45	28.5	0.464
	60	43.0	0.577
	80	44.0	0.442

TABLE II

THE EXCHANGE OF CYCLOPENTYL IODIDE WITH SODIUM IODIDE IN ETHANOL SOLUTION

Temp., °C.	Reactant concentration (molarity)		Maximum exchange, %	k(cc.$-$mole$^{-1}-$sec.$^{-1}$) ($\times 10^2$)
	RI	NaI		
Series A				
36.6	0.30	0.30	16	3.0
47.0	0.30	0.30	31	9.0
56.0	0.30	0.30	25	23.3
	0.20	0.40	37	24.4
64.0	0.45	0.15	26	47.2
	0.30	0.30	56	42.0
	0.15	0.45	43	42.5
	0.50	0.10	51	44.2
Series B				
36.6	0.30	0.30	19	3.3
50.7	0.30	0.30	37	14.1
56.0	0.30	0.30	36	26.9
64.0	0.30	0.30	70	55.6

TABLE III

SUMMARY OF RESULTS FOR THE EXCHANGE REACTIONS BETWEEN ALICYCLIC IODIDES AND SODIUM IODIDE IN ETHANOL SOLUTION

Alicyclic iodide	k(at 25°C) × 10^2 cc.$-$mole^{-1} $-$sec.$^{-1}$*	E** kcal/mole	log PZ**
Cyclopentyl iodide	0.88	21.0	13.4
Cyclohexyl iodide	0.0084	25.6	14.7
Cycloheptyl iodide	1.2	21.3	13.7
Cycloöctyl iodide	0.28	22.0	13.6
Cyclopentadecyl iodide	0.099	20.1	11.8
2-Heptyl iodide	0.77	21.8	13.9

* *Calculated from* $k = PZ\, e^{-E/RT}$, *using the values recorded for E and log PZ.*

** *Each value recorded is the average of the two values obtained for series A and series B experiments (except for cycloöctyl iodide, where only one limited series of experiments was possible). The probable error in the average values is approximately 3%.*

Discussion

The apparently anomalous behavior of a cyclohexyl derivative, referred to previously, appears to be confirmed in the present study. The considerably lower rate of exchange of cyclohexyl iodide is here accompanied by energy of activation and entropy of activation (log PZ) terms which differ considerably from those for the other compounds investigated.

There seems no good reason to believe that the carbon–iodine bond strength in cyclohexyl iodide is substantially greater than in the other alicyclic iodides. Presumably, then, the higher activation energy for the cyclohexyl iodide exchange is associated with the process of rendering coplanar the atoms at the site of reaction, prior to exchange and subsequent inversion. If it is assumed that the height of the energy barrier to be surmounted during exchange remains unaltered from member to member in the series, the greater activation energy may be attributed to the lower potential energy of the cyclohexyl ring initially (1, 7). The present results indicate that the extent of this stabilization of the cyclohexyl ring is about 4 kcal.

The larger entropy term for the cyclohexyl iodide exchange, compared with the values of log PZ for the other exchange reactions, is probably not anomalous. This is indicated by a plot of log PZ against the reciprocal of the number of carbon atoms in the organic iodide undergoing reaction, as in Fig 2. The figure includes results for the analogous exchange reaction with amyl iodide in ethanol (3). In the figure, a straight line has been drawn through the points but it is probable that the range of values involved represents the mid-portion of a sigmoid relation which rises rapidly as $\frac{1}{N}$ approaches zero and drops rapidly to a constant value of about 16 for small values of $\frac{1}{N}$ (3). Greater accuracy than that attained in the present work would be necessary to define the exact form of the relation.

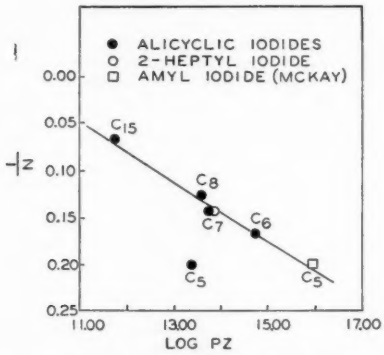

FIG. 2. *Inverse relation between number of carbon atoms and log PZ.*

The inverse relation between log *PZ* and the number of carbon atoms in the organic iodide may be accounted for if the entropy change associated with activation reflects a change in the number of equivalent constellations (1, 7), and this change has proportionately less effect as the number of possible equivalent constellations increases with increased ring size. The failure of cyclopentyl iodide to conform to the relation in Fig. 2 might be expected, since the cyclopentyl ring is approximately planar in the initial state (Fisher–Hirschfelder model) and relatively little change in it should occur as the atoms at the site of reaction are rendered coplanar during activation prior to exchange.

Acknowledgment

Grateful acknowledgment is made to the Committee on Research, McGill University, for financial assistance in the preparation of this manuscript.

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STUDIES OF RDX AND RELATED COMPOUNDS

III. THE REACTION TO FORM RDX FROM AMMONIUM NITRATE AND FORMALDEHYDE IN ACETIC ANHYDRIDE[1]

By A. Gillies, H. L. Williams, and C. A. Winkler

Abstract

This reaction at 35°C. exhibits a behavior indicative of the presence of an intermediate in the reaction. Reaction of paraformaldehyde and ammonium nitrate in glacial acetic acid resulted in the isolation of hexamine dinitrate. Evidence is presented to indicate that formation of hexamine dinitrate, accompanied by the production of nitric acid, is responsible for the production of RDX in the acetic anhydride system.

Introduction

Kinetic studies of the direct nitrolysis of hexamine with concentrated nitric acids to produce *cyclo*-trimethylenetrinitramine (RDX) have been reported in previous papers from this laboratory (6, 8). The present paper gives some of the results obtained by investigation of an alternative method of preparation involving the interaction of ammonium nitrate and paraformaldehyde in acetic anhydride (4, 7).

Experimental

The procedure, usually, was to place the desired amounts of ammonium nitrate and paraformaldehyde (expressed as moles of formaldehyde) in 125 ml. Erlenmeyer flasks, which were suspended in a thermostat from a shaker oscillating at about 140 cycles per minute. The temperature of the water bath was controlled to within $\pm$ 0.1°C. Acetic anhydride was then added, the time of its addition being taken as zero time for the reaction. The reaction was stopped, after the desired time interval, by the addition of about 20 ml. of water, the flask removed from the thermostat, and the mixture boiled for 15–20 min. After cooling, the flask was filled with water and allowed to stand, usually overnight. Precipitated RDX was collected in a sintered glass crucible, washed with water, dried in an air oven at 90°C. for two to three hours and weighed. In general, the weight of crude RDX was considered to be the yield and was calculated as per cent of the theoretical on a formaldehyde basis.

Results and Discussion

Presence of an Intermediate in the Reaction

RDX was produced only after an induction period, the length of which varied from two to six hours depending on the source of paraformaldehyde, when 0.02 mole paraformaldehyde, 0.04 mole ammonium nitrate, and 5 cc. acetic anhy-

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Quebec, with financial assistance from the National Research Council of Canada.

dride were allowed to react at 35°C. The presence of acetic or trichloracetic acid reduced the induction period somewhat, but neither acid had any appreciable effect on the rate of reaction following the induction period. It appeared from these results that an intermediate was formed during the reaction, the rate of its formation, but not the rate of its conversion to RDX, being influenced by the presence of acetic or trichloracetic acid.

A series of experiments was made in which the various reactants were individually withheld from reaction mixtures at 35°C. for a period equal to the induction period (3.5 hr.), followed by addition of the missing component and further reaction for 3.5 hr. No RDX was produced, hence no intermediate was formed, unless all three reactants were present.

The relative amounts of intermediate present at various times were determined as follows. Seven reaction mixtures, containing 13% acetic acid, were allowed to react for different times, after which they were filtered and six aliquots of each filtrate taken. The RDX content of one of these was determined by immediate dilution, that of the others after further periods of reaction at 35°C. The amounts of intermediate present at various times, expressed in terms of the amount of RDX it was capable of producing, are shown in Fig. 1. The top curve, for example, indicates the amount of intermediate present at the time of filtering, when filtration was made two to six hours after the experiment was started. The second curve from the top gives the amount of intermediate present one half hour after filtration when filtration was made at the same time intervals after start of the experiment; and similarly

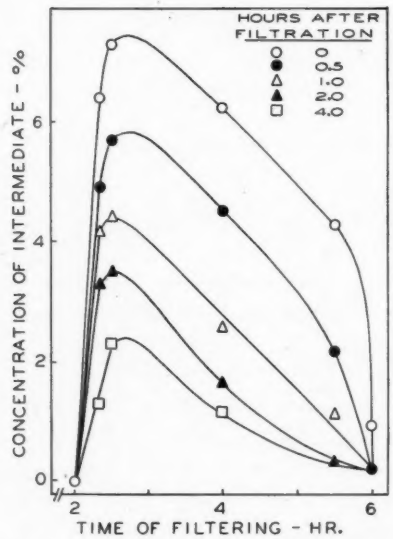

FIG. 1. *Relative amounts of intermediate present at various times.*

for the other curves. The results show that negligible amounts of intermediate were formed during the first two hours, while the maximum amount was present about 2.5 hr. after the start of the reaction. The RDX yield reached its maximum value at about six hours, after which time the amount of intermediate present was negligible.

The activation energy for conversion of the intermediate to RDX was determined by allowing paraformaldehyde and ammonium nitrate to react to the end of the induction period (2.5 hr.) at 35°C. in 7.5 cc. acetic anhydride containing 13% acetic acid, after which the mixtures were filtered and the filtrates allowed to react further at 25°, 30°, 35°, and 40°C. From initial rates, an activation energy of roughly 18.5 kcal. was obtained.

Using the halochromic compounds of Conant and Hall (3), it was possible to estimate, despite rapid fading of the indicators, that the pH (HAc) of a normal reaction mixture started at about zero but changed to a value of about − 3 as reaction progressed. Comparison with a pH (HAc) value of − 3.2 determined for sulphuric acid (one drop) in acetic acid (5 cc.) containing acetic anhydride gave clear indication that a strong acid, as well as the intermediate discussed above, was formed during reaction.

When paraformaldehyde (0.02 mole) and ammonium nitrate (0.04 mole) were allowed to react in glacial acetic acid (6 cc.) for 11 hr. at 35°C., followed by addition of acetic anhydride (6 cc.) and further reaction for one hour, production of RDX occurred without an induction period following the addition of anhydride. It appeared from this that the intermediate had been formed by reaction of paraformaldehyde and ammonium nitrate in acetic acid. The rate of conversion to RDX of intermediate so formed was increased by the addition of a few drops of sulphuric or perchloric acid, while the yield of RDX obtained was adversely affected by the presence of formaldehyde in excess of a mole ratio of approximately 1.5:1 with ammonium nitrate.

The activation energy for the formation of RDX subsequent to the addition of anhydride to a glacial acetic acid preparation of the intermediate was found to be about 19.5 kcal. Comparison of this value with that recorded previously for conversion, also in acetic acid – acetic anhydride media, of the intermediate present in the filtrates from reaction mixtures suggests that the same substance is involved in the two cases.

When paraformaldehyde and ammonium nitrate reacted at 35°C. in glacial acetic acid, it was observed that the mixture gradually cleared as the paraformaldehyde reacted, until at the end of 2.5 hr. only excess ammonium nitrate remained. An hour later there appeared a dense white precipitate. The excess ammonium nitrate was removed by further reaction with a slight excess of paraformaldehyde, the precipitate filtered off, washed with glacial acetic acid and acetone, and dried in a vacuum desiccator. Elemental analysis gave*: C, 27.4, 27.5; H, 5.3, 5.4; N, 30.7, 30.8%. For hexamine dinitrate, C, 27.1;

*We are indebted to Dr. G. F Wright, University of Toronto, for the analyses.

H, 5.3; N, 31.6%. The precipitate gave an X-ray powder photograph which showed only the characteristic pattern for hexamine dinitrate. The formation of hexamine by reaction of formaldehyde and ammonia in acidic media has been reported previously by Baur and Ruetschi (2).

Isolation of hexamine dinitrate from the paraformaldehyde – ammonium nitrate reaction in glacial acetic acid, and comparison of the behavior of this system in the presence of acetic anhydride with the characteristics of the reaction to produce RDX from paraformaldehyde, ammonium nitrate, and acetic anhydride, give strong indication that hexamine dinitrate is the intermediate formed during the latter reaction. On this basis, the observed deleterious effect of excess paraformaldehyde on RDX yield may be readily explained, since hexamine is decomposed by formaldehyde in glacial acetic acid at a rate which increases with increased mole ratio of formaldehyde to hexamine (5).

Nitric acid is undoubtedly the strong acid revealed by the Conant – Hall indicators to be formed in the paraformaldehyde – ammonium nitrate – acetic anhydride reaction mixtures. Formation of nitric acid simultaneously with hexamine dinitrate in the reaction mixtures must be assumed to account for the nitrolysis reaction to produce RDX, unless the presence of another intermediate capable of promoting nitrolysis can be demonstrated. Rather exhaustive examination of the system failed to indicate any such alternative agent for nitrolysis.

While one of the functions of acetic anhydride is almost certainly to remove water formed during the initial production of hexamine and nitric acid, and thus maintain the nitric acid concentration at a high level favorable for nitrolysis (7), it seems likely that it also has the role of a "fast" solvent for the reaction. This was revealed by studies in which 6 cc. of various solvents were added to 3 cc. of filtrate obtained from the reaction of ammonium nitrate and paraformaldehyde in acetic acid. The solvent – filtrate mixture was allowed to react with 3 cc. acetic anhydride for 30 min. at 35°C., after which 0.5 gm. hexamine dinitrate was added. The rates of production of RDX, determined in the usual manner, were in the following order for the solvents listed: acetic anhydride $\gg$ propionic anhydride $>$ nitromethane $>$ acetic acid $>$ acetonitrile $>$ benzene $>$ propionic acid $\gg$ dioxane.

Since the present studies gave strong indication that the paraformaldehyde – ammonium nitrate – acetic anhydride reaction to produce RDX consists essentially of the production of hexamine followed by its nitrolysis, investigation of this system was abandoned in favor of studies on the Bachmann reaction (1) in which hexamine and nitric acid are among the starting materials. The results of several studies with the Bachmann system will be reported in subsequent papers.

Acknowledgment

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PREPARATION AND NITRATION OF 1-SUBSTITUTED-2-NITRAMINO-2-IMIDAZOLINES[1]

By A. F. McKay, J. R. G. Bryce, and D. E. Rivington[2]

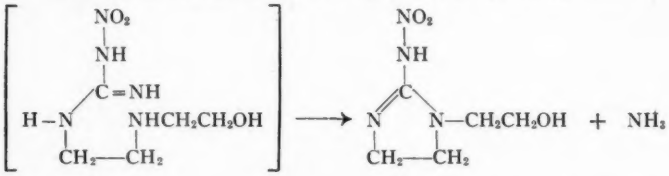
Abstract

A series of 1-substituted-2-nitramino-2-imidazolines have been prepared and nitrated in mixed-acid and nitric acid – acetic anhydride media. These compounds gave the corresponding 1-substituted-2-nitrimino-3-nitroimidazolidines which proved to be quite stable. The hydrolysis products of some of these compounds are described.

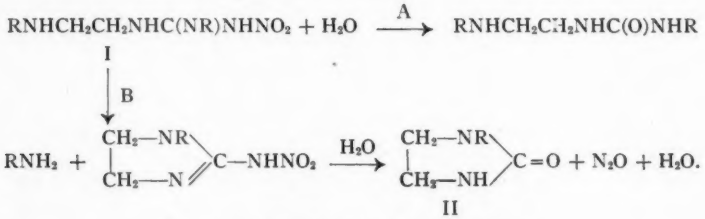
The nitration studies (5, 8) on 2-nitramino-2-imidazoline have been extended to 1-substituted-2-nitramino-2-imidazolines. The latter compounds on nitration give a new series of compounds, viz., 1-substituted-2-nitrimino-3-nitroimidazolidines.

The compounds employed in the present investigation were prepared by the addition of 1-amino-3-azabutane, 1-amino-3-azapentane, 1-amino-5-hydroxy-3-azapentane, 1, 5-diamino-3-azapentane, and 1, 8-diamino-3, 6-diazaoctane respectively to N-methyl-N-nitroso-N′-nitroguanidine (4, 6, 7). In all cases, cyclizations occurred to give 1-substituted-2-nitramino-2-imidazolines. The reaction of 1-amino-5-hydroxy-3-azapentane with methylnitrosonitroguanidine may be represented as follows:

$$HOCH_2CH_2NHCH_2CH_2NH_2 + CH_3N(NO)C(NH)NHNO_2 \longrightarrow$$



A similar cyclization reaction (B) involving a secondary amino group also explains the formation of 1-aryl-2-imidazolidones (II) from 1-β-arylaminoethyl-2-aryl-3-nitroguanidines (I) on alkaline hydrolysis (9).



R = phenyl, ρ-tolyl, ρ-anisyl or ρ-phenetyl

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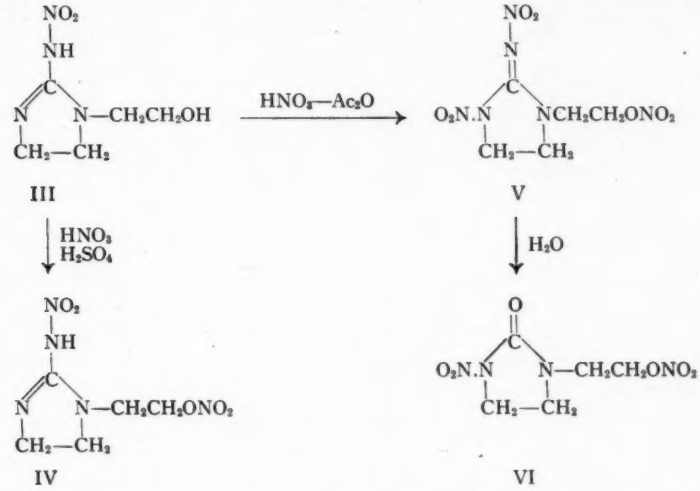
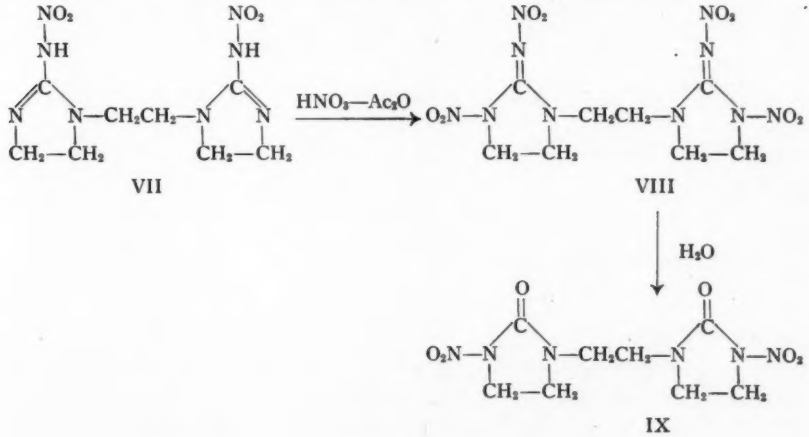
1-β-Hydroxyethyl-2-nitramino-2-imidazoline (III) on treatment with mixed-acid gave 1-β-nitroxyethyl-2-nitramino-2-imidazoline. On the other hand, nitration in acetic anhydride – nitric acid medium gave a 94% yield

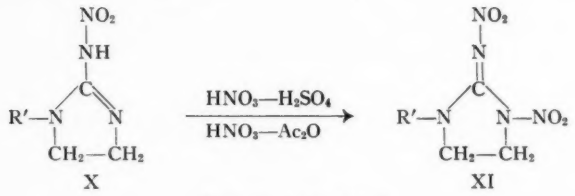


of 1-β-nitroxyethyl-2-nitrimino-3-nitroimidazolidine (V). The structure of the latter compound (V) was confirmed by analysis and its hydrolysis in water to 1-β-nitroxyethyl-3-nitro-2-imidazolidone (VI).

Nitration of *bis*-1-(2-nitramino-2-imidazolinyl) ethane (VII) in nitric acid—acetic anhydride medium gave *bis*-1-(2-nitrimino-3-nitroimidazolidinyl) ethane (VIII) in 84–96% yield. This compound (VIII) on boiling with water hydrolyzed to *bis*-1-(3-nitro-2-imidazolidonyl) ethane (IX).

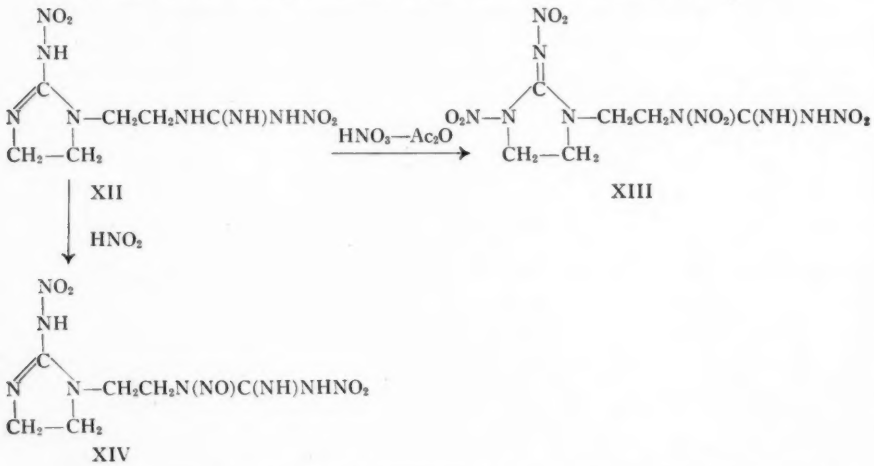


1-Methyl-(X, R′ = Me) and 1-ethyl-2-nitramino-2-imidazoline (X, R′ = Et) behaved differently from the above compounds in that both mixed-acid and nitric acid – acetic anhydride media gave the corresponding 1-alkyl-2-nitrimino-3-nitroimidazolidines (XI).



R′ = methyl or ethyl

1-β-Nitroguanylaminoethyl-2-nitramino-2-imidazoline (XII) on treatment with absolute nitric acid – acetic anhydride yields besides 1-(N-nitroguanyl-N-nitro-β-aminoethyl)-2-nitrimino-3-nitroimidazolidine (XIII) another compound as yet unidentified. This is the second example in which an additional nitro group has been introduced into a linear substituted nitroguanidine structure. The first one to be described (6) was N-β-nitroxyethyl-N-nitro-N′-nitroguanidine. On nitrosation, 1-β-nitroguanylaminoethyl-2-nitramino-2-imidazoline (XII) yields 1-(N-nitroguanyl-N-nitroso-β-aminoethyl)-2-nitramino-2-imidazoline (XIV). The position of the nitroso group was verified by treating with benzylamine when a 75% yield of benzylnitroguanidine was obtained. If the nitroso group had been present on the ring nitrogen, more highly substituted nitroguanidines would have been obtained (9).



All the above 1-substituted-2-nitramino-2-imidazolines give stable nitrimine compounds. When there is no substituent present in position 1, the 2-nitramino-2-imidazolines on nitration with excess nitric acid in the presence of

acetic anhydride are converted into the corresponding 1, 3-dinitro-2-imidazolidones (5, 8), the nitramino group of position 2 being eliminated as N_2O.

The Franchimont test (2) for nitramines has proved very useful in the study of the derivatives of nitroguanidine. Some of these compounds and the color developed with dimethylaniline and diethylaniline are listed in Table I.

TABLE I

Franchimont nitramine test

Compound	Diethyl aniline	Dimethyl aniline
Methylnitroguanidine (6)	Light green	Negative
Allylnitroguanidine (3)	"	"
Cyclohexylnitroguanidine (3)	Negative	Pink
p-Tolylnitroguanidine (3)	"	"
m-Tolylnitroguanidine (3)	Green	"
o-Tolylnitroguanidine (3)	Green	"
2, 4-Dimethylphenylnitroguanidine (3)	Negative	Pink
p-tert. Amylphenylnitroguanidine (3)	Green	Negative
dl-α-Phenylethylnitroguanidine (3)	Negative	Pink
Phenylnitroguanidine (3)	Deep green	Deep green
1-β-Nitroxyethyl-2-nitrimino-3-nitroimidazolidine	Green (fades)	Pink
1-Methyl-2-nitrimino-3-nitroimidazolidine	"	"
1-Ethyl-2-nitrimino-3-nitroimidazolidine	Pink	"
1-Methyl-2-nitramino-2-imidazoline	"	"
1-Ethyl-2-nitramino-2-imidazoline	"	"
1-β-Hydroxyethyl-2-nitramino-2-imidazoline	"	"
1-*p*-Anisyl-2-nitramino-2-imidazoline (9)	Deep green	Deep green
1, 3-Dinitro-4-methyl-2-hexahydropyrimidone (4)	"	"
2, 6-Dinitro-2, 6-diazacycloheptanone-1 (7)	"	"
2-Nitramino-5-nitroxy-2-tetrahydropyrimidine (4)		

These data show the differences to be expected with the two reagents. A summary of the behavior of several types of nitramines in the Franchimont test, which is based on the observations employing more than 60 compounds, is presented in Table II. The procedure used in these tests, along with further observations, is described in an earlier paper (3).

TABLE II

Franchimont color test

Class of compounds	Dimethyl aniline	Diethyl aniline
1. N-alkyl-N′-nitroguanidines	Negative	Pale green
2. N-Aralkyl-N′-nitroguanidines	Negative	Pale green
3. N-Aryl-N′-nitroguanidines	Pink	Negative or pale green
4. 2-Nitramino-2-imidazolines[1]	Pink	Pink
5. 1, 3-Dinitro-1, 3-diaza-2-cycloalkanones	Deep green	Deep green
6. 1-Nitro-2-amino-2-imidazolines	"	"

[1]*Nitroxy derivatives, e.g., 1-β-nitroxyethyl-2-nitramino-2-imidazoline, give deep green colors with both dimethyl and diethylaniline.*

Experimental*

N-Methyl- and N-Ethylethylenediamine

N-Methyl- and N-ethylethylenediamine were prepared by the method of Aspinall (1) in 10 and 20% yields respectively.

1-*Methyl-2-nitramino-2-imidazoline*

Eleven and one-half grams (0.156 mole) of N-methylethylenediamine was added to a suspension of 23.2 gm. (0.157 mole) of methylnitrosonitroguanidine in 45 cc. of water. The temperature of the reaction mixture was maintained at 22–25°C. during the addition of the amine, which required 20 min. The clear solution was boiled for seven minutes and then placed in an evaporating dish. After most of the water had evaporated, a pale yellow oil remained. This oil yielded white flat needlelike crystals on standing overnight in the refrigerator. These crystals were filtered from the oil and washed with water. An equal volume of water was added to the oily filtrate and the solution was boiled for five minutes. An additional 1.1 gm. of product was obtained by repeating the above procedure. The total yield of product melting at 110–113°C. was 8.2 gm. (36.2%). One crystallization from absolute ethanol (2.42 cc. per gm.) raised the melting point to 115–116°C. Calc. for $C_4H_8N_4O_2$: C, 33.32; H, 5.52; N, 38.9%. Found: C, 33.26; H, 5.64; N, 38.30%.

1-*Ethyl-2-nitramino-2-imidazoline*

When 11.3 gm. (0.068 mole) of methylnitrosonitroguanidine was treated with 5.7 gm. (0.065 mole) of N-ethylethylenediamine under the conditions described above, 4.2 gm. (40.8% of theory) of crude product was obtained. The melting point of the product was raised from 78–83°C. to 86.5°C. by one crystallization from absolute ethanol (1.9 cc. per gm.). Calc. for $C_5H_{10}N_4O_2$: C, 37.95; H, 6.32; N, 35.4%. Found: C, 38.15; H, 6.18; N, 35.12%.

1-β-*Hydroxyethyl-2-nitramino-2-imidazoline*

1-Amino-5-hydroxy-3-azapentane (18.7 gm.; 0.18 mole) was added portionwise with stirring to a suspension of 25 gm. (0.17 mole) of N-methyl-N-nitroso-N′-nitroguanidine in 50 cc. of water. During the addition of the amine, which required 35 min. the temperature was held at 20–22°C. The clear solution was then evaporated on a hot-plate to one-half its original volume and placed in the refrigerator overnight. The crystals were removed by filtration and washed with 95% ethanol. On evaporation of the filtrate to 1/3 its original volume and cooling, a second crop of crystals was obtained. The total yield of product (m.p. 129–131.5°C.) was 12.6 gm. (42%). After crystallization from 95% ethanol, the melting point was 131.5–132°C. Calc. for $C_5H_{10}N_4O_3$: C, 34.5; H, 5.75; N, 32.15%. Found: C, 34.6; H, 5.57; N, 32.02%.

**All melting points are uncorrected.*

1-β-Nitroguanylaminoethyl-2-nitramino-2-imidazoline

1, 5-Diamino-3-azapentane added to two mole equivalents of methylnitrosonitroguanidine under the conditions described above for 1-β-hydroxyethyl-2-nitramino-2-imidazoline gave a 45% yield of 1-β-nitroguanylaminoethyl-2-nitramino-2-imidazoline (m.p. 195–197°C. with decomposition). One crystallization from water (30 cc. per gm.) gave crystals melting at 197°C. with decomposition. Calc. for $C_6H_{12}N_8O_4$: C, 27.69; H, 4.61; N, 43.09%. Found: C, 27.69; H, 4.81; N, 43.06%.

Bis-1-(2-*nitramino*-2-*imidazolinyl*) *ethane*

The reaction of 1, 8-diamino-3, 6-diazaöctane with 2 mole equivalents of methylnitrosonitroguanidine as described above gave a 43.4% yield of *bis*-1-(2-nitramino-2-imidazolinyl) ethane melting at 300–301°C. with decomposition. Calc. for $C_8H_{14}N_8O_4$: C, 33.6; H, 4.90; N, 39.1%. Found: C, 33.6; H, 5.06; N, 38.7%.

1-β-Nitroxyethyl-2-nitramino-2-imidazoline

1-β-Hydroxyethyl-2-nitramino-2-imidazoline (930 mgm.; 0.0053 mole) was added to a solution of 2.1 cc. (0.06 mole) of absolute nitric acid in 3.2 cc. (0.06 mole) of sulphuric acid (sp. gr. 1.84) at −5°C. The reaction mixture was stirred at this temperature for 20 min. after which the clear solution was poured onto ice. A crystalline product separated which was filtered off and washed with water. It melted at 110–115°C., yield 855 mgm. (73.8%). One crystallization from methanol (11.8 cc. per gm.) raised the melting point to 114.8–115.2°C. Calc. for $C_5H_9N_5O_5$: C, 27.4; H, 4.11; N, 31.95%. Found: C, 27.8; H, 4.17; N, 31.92%.

1-β-Nitroxyethyl-2-nitrimino-3-nitroimidazolidine

A solution of 9.24 cc. (0.22 mole) of absolute nitric acid in 20.6 cc. (0.22 mole) of acetic anhydride was prepared at 0°C. To this solution 3.88 gm. (0.022 mole) of 1-β-hydroxyethyl-2-nitramino-2-imidazoline was added portionwise over a period of five minutes. The temperature of the reaction mixture was maintained at 34°C. for a period of 25 min. and then the contents of the flask was poured onto ice (50 gm.), the crystals recovered by filtration and washed with water. The product melted at 114.5–115.5°C. with decomposition, yield 5.55 gm. (94.2%). One crystallization from methanol (61.9 cc. per gm.) raised the melting point to 115–116°C. with decomposition. Calc. for $C_5H_8N_6O_7$: C, 22.71; H, 3.04; N, 31.8%. Found: C, 22.85; H, 3.16; N, 31.3%.

1-β-Nitroxyethyl-2-nitrimino-3-nitroimidazolidine combined violently with phenylhydrazine giving a partially charred mass. The reaction in methanol gave first a deep green color similar to the Franchimont nitramine test (2) with dimethylaniline and then gas was evolved and the solution turned red. All attempts to obtain a crystalline product were unsuccessful.

1-β-Nitroxyethyl-3-nitro-2-imidazolidone

One gram (0.0038 mole) of 1-β-nitroxyethyl-2-nitrimino-3-nitroimidazolidine in 20 cc. of water was refluxed for one and a half hours. On cooling to room temperature, a crystalline solid separated. These crystals (m.p. 101–102°C.) were filtered off and washed with water, yield 485 mgm. (58.3%). The melting point remained unchanged after one crystallization from water (34 cc. per gm.). Calc. for $C_5H_8N_4O_6$: C, 27.26; H, 3.64; N, 25.40%. Found: C, 27.60; H, 3.65; N, 25.30%.

Bis-1-(2-nitrimino-3-nitroimidazolidinyl) ethane

Nitration of *bis*-1-(2-nitramino-2-imidazolinyl) ethane under the conditions described above for the preparation of 1-β-nitroxyethyl-2-nitrimino-3-nitroimidazolidine with the exception that the reaction temperature was maintained at 50–56°C. gave a 96% yield of *bis*-1-(2-nitrimino-3-nitroimidazolidinyl) ethane (m.p. 177–178°C. with decomposition). This product was insoluble in all organic solvents and water. On solution in hot 70% nitric acid and dilution with water no water insoluble material separated. Another run was carried out at 40°C. in the presence of 1/20 mole equivalent of zinc chloride. Under the latter conditions an 84% yield of the nitrimino compound was obtained melting at 180–181°C. with decomposition. Calc. for $C_8H_{12}N_{10}O_8$: C, 25.55; H, 3.19; N, 37.24%. Found: C, 25.85; H, 3.26; N, 37.0%.

Bis-1-(3-nitro-2-imidazolidonyl) ethane

Bis-1-(2-nitrimino-3-nitroimidazolidinyl) ethane (990 mgm.; 0.0026 mole) in 40 cc. of water was refluxed for three hours. After cooling to room temperature the crystals were filtered off and washed with water. The product melted at 242–243°C. with decomposition. Since this compound was practically insoluble in all solvents tried, it was analyzed without further purification. Calc. for $C_8H_{12}N_6O_6$: C, 33.33; H, 4.17; N, 29.15%. Found: C, 33.31; H, 4.19; N, 29.03%.

1-Methyl-2-nitramino-3-nitroimidazolidine

A. *Acetic Anhydride - Nitric Acid Nitration*

The nitrating solution was prepared by adding 6.0 gm. (0.143 mole) of absolute nitric acid to 13.3 cc. (0.143 mole) of acetic anhydride at 0°C. Two grams (0.014 mole) of 1-methyl-2-nitramino-2-imidazoline was added over a period of five minutes. Then the temperature of the stirred reaction mixture was raised to 32°C. and maintained at this level for 25 min. The reaction mixture was poured onto ice and the white solid removed by filtration and washed with water, yield 2.16 gm. (91% of theory). The melting point of 167–168°C. with decomposition was raised to 169–170°C. with decomposition after one crystallization from absolute methanol (270 cc. per gm.). Calc. for $C_4H_7N_5O_4$: C, 25.38; H, 3.70; N, 37.0%. Found: C, 25.27; H, 4.01; N, 36.75%.

B. *Mixed-acid Nitration*

A solution of 5.5 cc. (0.16 mole) of absolute nitric acid in 8.4 cc. (0.16 mole) of sulphuric acid (sp. gr. 1.84) was cooled to −5°C. and 2.0 gm. (0.014 mole) of 1-methyl-2-nitramino-2-imidazoline was added within five minutes. The reaction mixture was stirred at −5°C. for 20 min. and then poured onto ice. The white solid was removed by filtration and washed with water, yield 0.46 gm. (17.5% of theory). After crystallizing from methanol the melting point was raised from 165–168°C. to 169–170°C., with decomposition. This product did not depress the melting point of 1-methyl-2-nitrimino-3-nitroimidazolidine (m.p. 169–170°C. with dec.) prepared from the nitric acid – acetic anhydride nitration of 1-methyl-2-nitramino-2-imidazoline.

1-*Ethyl*-2-*nitrimino*-3-*nitroimidazolidine*

1-Ethyl-2-nitramino-2-imidazoline (1 gm.; 0.0064 mole) was nitrated under the conditions described in method A above for the preparation of 1-methyl-2-nitrimino-3-nitroimidazolidine. The yield was 1.1 gm. or 86% of theory. The crude product melted at 136–137°C. with decomposition. This melting point was raised to 137–138°C. with decomposition by one crystallization from absolute methanol (121 cc. per gm.). Calc. for $C_5H_9N_5O_4$: C, 29.55; H, 4.43; N, 34.5%. Found: C, 29.71; H, 4.68; N, 34.0%.

Mixed-acid nitration of 1-ethyl-2-nitramino-2-imidazoline as above gave a 28% yield of 1-ethyl-2-nitrimino-3-nitroimidazolidine melting at 137–138°C. with decomposition alone and on admixture with an authentic sample of 1-ethyl-2-nitrimino-3-nitroimidazolidine.

1-(*N-nitroguanyl-N-nitro-β-aminoethyl*)-2-*nitrimino*-3-*nitroimidazolidine*

To a solution of 16.1 cc. (0.384 mole) of absolute nitric acid in 36.1 cc. (0.384 mole) of acetic anhydride was added 10.0 gm. (0.0384 mole) of 1-β-nitroguanylaminoethyl-2-nitramino-2-imidazoline over a period of six minutes. During the addition period the temperature rose from 0 to 26.8°C. and a colorless gas was evolved. This mixture was allowed to come to room temperature, after which it was poured onto 100 gm. of ice. The solid was recovered by filtration, washed with water and air dried, yield 7.9 gm. The melting point of 134–139°C. with decomposition of the crude product was raised to constant value of 161–162°C. with decomposition by one crystallization from absolute methanol (100 cc. per gm.), yield 2.09 gm. (16.0%). Calc. for $C_6H_{10}N_{10}O_8$: C, 20.58; H, 2.85; N, 40.00%. Found: C, 20.30; H, 3.00; N, 39.52%.

On evaporation of the filtrate from the above crystallization a second compound (1.24 gm.) was obtained which melted at 180.5–181.5°C. with decomposition. The latter compound has not been identified at present.

1-(*N-Nitroguanyl-N-nitroso-β-aminoethyl*)-2-*nitramino*-2-*imidazoline*

1-β-Nitroguanylaminoethyl-2-nitramino-2-imidazoline (2.5 gm.; 0.0096 mole) was dissolved in 15 cc. of nitric acid (sp. gr. 1.42) and 15 cc. of water added.

The clear solution was maintained at 8–10°C., while 1.75 gm. (0.026 mole) of sodium nitrite in 10 cc. of water was added over a period of 10 min. Then the solution was stirred while the temperature was allowed to rise to 18°C. and a yellow crystalline precipitate formed. The yellow nitrosamine was washed with water and methanol (20 cc.) after filtration. It melted at 176°C. with decomposition, yield 1.54 gm. (55.6%). Calc. for $C_6H_{11}N_9O_5$: C, 25.29; H, 3.82; N, 42.85%. Found: C, 25.21; H, 3.72; N, 42.88%.

A sample of 1-(N-nitroguanyl-N-nitroso-β-aminoethyl)-2-nitramino-2-imidazoline on treatment with benzylamine under the conditions employed with the nitrosamines of this series (4, 6) gave a 75% yield of benzylnitroguanidine (m.p. 182–183°C.).

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PREPARATION OF SUBSTITUTED NITROGUANIDINES AND THE CYCLIZATION OF N-γ-NITROXYBUTYL-N′-NITROGUANIDINE[1]

By A. F. McKay and H. P. Thomas

Abstract

Several new substituted nitroguanidines were prepared by the reaction of amines with N-methyl-N-nitroso-N′-nitroguanidine. N-γ-Nitroxybutyl-N′-nitroguanidine was cyclized to 1-nitro-2-amino-6-methyl-Δ^2-tetrahydropyrimidinium nitrate by refluxing in water.

A further series of nitroguanidine derivatives have been prepared by the reaction of primary amines with N-methyl-N-nitroso-N′-nitroguanidine (3, 6). These are listed in Table I.

The compounds possessing structure I were insoluble in the common

$$O_2NNHC(NH)NH(CH_2)_nNHC(NH)NHNO_2$$

I

$n = 2 - 4$

organic solvents and water. They did not nitrosate under the usual conditions (3, 5, 6). Generally these compounds were recovered unchanged from the nitrosating medium on dilution with water.

The diaminotoluenes combined with only one equivalent of N-methyl-N-nitroso-N′-nitroguanidine to give N-aminomethylphenyl-N′-nitroguanidines. This would be expected from the previous observation that the rate of reaction in aqueous ethanol is directly proportional to the K_b of the amine (3). The second amino group would have a much lower basicity constant and a much lower rate of reaction. This posed the problem of determining the position of the amino group involved in the above reaction.

The observation was made previously (8) that N-*o*-methylphenyl-N′-nitroguanidine as well as other phenyl nitroguanidine derivatives having a methyl group ortho to the attachment of the nitroguanyl group gave a green color in the Franchimont test (1) using diethylaniline. The nitroguanyl derivatives of 2,4-diaminotoluene and 2,5-diaminotoluene gave no green color, so it was assumed that the positions of the nitroguanyl group were 4 and 5 respectively. This was confirmed experimentally by diazotization of the free amino group of the 2,4-diaminotoluene derivative (II). The diazonium chloride (III) was converted to the salt of 1, 5-naphthalene disulphonic acid by the method of Hodgson and Marsden (2). Then the crystalline double salt was suspended in ethanol and zinc dust added. At the end of the reaction a 10% yield of N-*p*-methylphenyl-N′-nitroguanidine (IV) was

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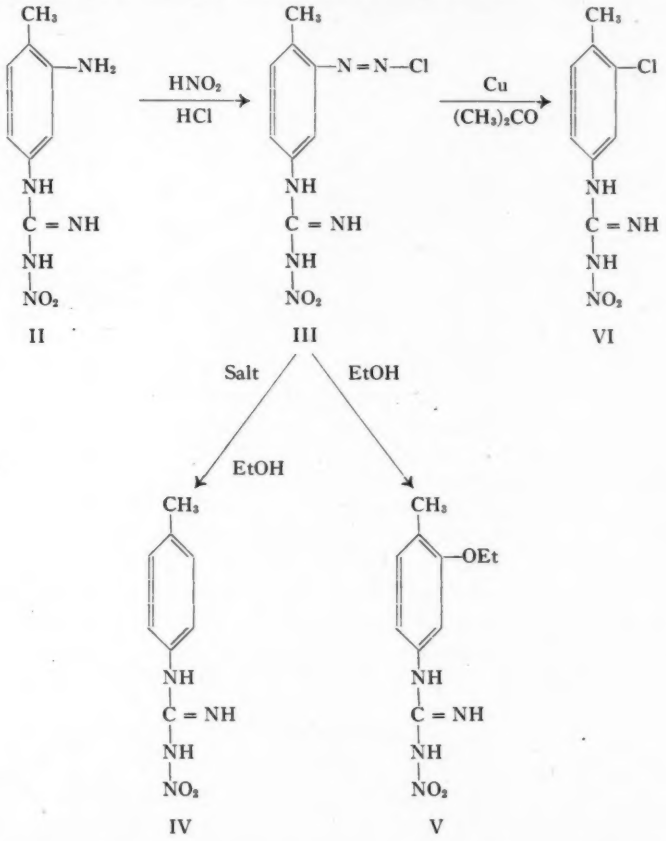
TABLE I

SUBSTITUTED NITROGUANIDINES

Compound	M.p., °C.(a)	Yield, %	Analyses, %					
			Calc. C	Found C	Calc. H	Found H	Calc. N	Found N
N-*p*-Hydroxyphenyl-N′-nitroguanidine	236	90.0	42.8	43.02	4.08	4.19	28.58	28.61
N-*m*-Hydroxyphenyl-N′-nitroguanidine	177–178	66.5	42.8	43.09	4.08	4.00	28.58	28.10
N-*p*-Diethylaminophenyl-N′-nitroguanidine	196	79.7	52.6	52.57	6.77	6.59	27.90	28.21
N-4-Amino-3-methylphenyl-N′-nitroguanidine	201–202	83.0	45.8	46.03	5.30	5.35	33.45	33.12
N-3-Amino-4-methylphenyl-N′-nitroguanidine	178–179.5	81.2	45.8	45.92	5.30	5.33	33.45	33.55
1, 2-Di-(nitroguanylamino) ethane*	248–248.5	75.3	20.5	20.76	4.27	4.38	47.83	47.36
1, 3-Di-(nitroguanylamino)propane*	234–235	77.5	24.2	23.90	4.84	5.08	45.14	44.80
1, 3-Di-(nitroguanylamino)butane*	230	16.0	27.47	27.40	5.38	5.57	42.75	42.65
1, 4-Di-(nitroguanylamino)butane**	>265	25.0	27.47	27.44	5.38	5.80	—	—

a. All compounds melted with decomposition.
* *Prepared by D. E. Rivington, Queen's University, Kingston, Ontario.*
** *This compound was obtained as a by-product in the preparation of 2-nitramino-1, 3-diazacycloheptene-2 (4).*

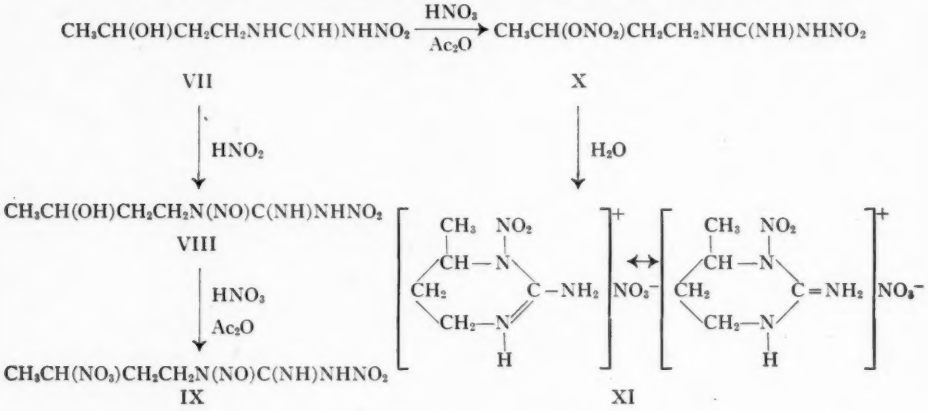
obtained. This verified the above conclusion that the compound derived from the reaction of 2,4-diaminotoluene with N-methyl-N-nitroso-N′-nitroguanidine was N-3-amino-4-methylphenyl-N′-nitroguanidine (II). It was concluded also that with 2,5-diaminotoluene, the 5-amino group was the one involved in the reaction with methylnitrosonitroguanidine.



During the investigation of methods for removal of the free amino group in N-3-amino-4-methylphenyl-N′-nitroguanidine (II) the diazonium chloride (III) was heated with ethanol and with copper in acetone. In the former case N-3-ethoxy-4-methylphenyl-N′-nitroguanidine (V) was obtained. In the latter case with copper in acetone a normal Gattermann reaction occurred yielding the corresponding N-3-chloro-4-methylphenyl-N′-nitroguanidine (VI).

It was demonstrated recently (5) that certain N-β-substituted ethyl-N′-nitroguanidines cyclized on heating to give heterocyclics having two ring nitrogens. Therefore we were interested in extending these observations to

include the N-γ-substituted propyl-N′-nitroguanidines. The starting material, N-γ-hydroxybutyl-N′-nitroguanidine (VII), was obtained as an oil by adding N-methyl-N-nitroso-N′-nitroguanidine to an aqueous solution of 1-amino-3-hydroxybutane. The oily product was characterized by converting



it to N-γ-hydroxybutyl-N-nitroso-N′-nitroguanidine (VIII) for analyses. This latter product on nitration in absolute nitric acid – acetic anhydride medium gave N-γ-nitroxybutyl-N-nitroso-N′-nitroguanidine (IX). After characterization, the original oil (VII) was nitrated in nitric acid – acetic anhydride medium to yield N-γ-nitroxybutyl-N′-nitroguanidine (X). On refluxing this compound (X) in water it cyclized to 1-nitro-2-amino-6-methyl-Δ^2-tetrahydropyrimidinium nitrate (XI) in 72% yield. The cyclized product (XI) gave a positive test (10) for nitrate ion with Nitron and a crystalline picrate. Thus the rearrangement discovered (5) with N-β-substituted ethyl-N′-nitroguanidine also occurs with N-γ-nitroxybutyl-N′nitroguanidine (X).

Experimental*

Substituted Nitroguanidines

The substituted nitroguanidines described in this paper were prepared by adding 3 mole equivalents of the amine to 1 mole equivalent of N-methyl-N-nitroso-N′-nitroguanidine in 50% aqueous ethanol as previously described (3). These compounds are tabulated in Table I.

1, 2-Di-(nitroguanylamino)ethane and Homologues

1, 2-Di-(nitroguanylamino)ethane and homologues were prepared by adding 1 mole equivalent of amine to 2 mole equivalents of N-methyl-N-nitroso-N′-nitroguanidine in methanol as described above. The products were insoluble in all common solvents and were purified by extraction with 95% ethanol or water. The final products are described in Table I.

**All melting points are uncorrected.*

1, 4-*Di-(nitroguanylamino)butane*

When 2-nitramino-1, 3-diazacycloheptene-2 was prepared by the method of McKay and Wright (7) from 0.038 mole of nitroguanidine and 0.04 mole of 1, 4-diaminobutane dihydrochloride, 4 gm. of a crude product melting at 200–201°C. with decomposition was obtained. This product has now been separated into two constituents by extraction with 200 cc. of acetone. The acetone extract contained 1.8 gm. (29.6%) of 2-nitramino-1, 3-diazacycloheptene-2 (m.p. 219–220°C. with decomposition). The acetone insoluble portion (1.5 gm.; 25%) did not melt below 265°C.; instead, it charred. It proved to be the linear 1, 4-di-(nitroguanylamino)butane (cf. Table I).

Deamination of N-3-Amino-4-methylphenyl-N′-nitroguanidine

The diazonium chloride of 4.0 gm. (0.02 mole) of N-3-amino-4-methylphenyl-N′-nitroguanidine was prepared by the method of Wallingford and Krueger (9), omitting the sodium hydroxide treatment. It was then converted to the 1, 5-naphthalene disulphonic-acid salt by the method of Hodgson and Marsden (2). The crystalline salt (6.9 gm.) was added to 50 cc. of ethanol and stirred mechanically at room temperature while 1.3 gm. of zinc dust was added portionwise. The stirring was continued for 11 hr., after which the precipitate was removed by filtration and the filtrate evaporated to 5 cc. A gummy product (0.67 gm.) was obtained which melted at 157°C. after one crystallization from ethanol, yield 0.26 gm. (10%). After several crystallizations from ethanol the product melted at 164°C. and it did not depress the melting point of an authentic sample of N-*p*-methylphenyl-N′-nitroguanidine (m.p. 165–166°C.).

N-3-Ethoxy-4-methylphenyl-N′-nitroguanidine

A sample of N-3-diazonium chloride-4-methylphenyl-N′-nitroguanidine prepared from 2 gm. (0.01 mole) of N-3-amino-4-methylphenyl-N′-nitroguanidine as described above was stirred with 100 cc. of ethanol at 45°C. for two hours. The solution was concentrated to a volume of 50 cc. and the precipitate removed by filtration. This precipitate was washed with ether, leaving a residue of 0.6 gm. (25%) which melted at 170–180°C. Two crystallizations from ethanol raised the melting point of the N-3-ethoxy-4-methylphenyl-N′-nitroguanidine to 185. 5–186°C. Calc. for $C_{10}H_{14}N_4O_3$: C, 50.50; H, 5.92; N, 23.52%. Found: C, 50.55; H, 6.14; N, 23.71%.

N-3-Chloro-4-methylphenyl-N′-nitroguanidine

N-3-Diazonium chloride-4-methylphenyl-N′-nitroguanidine, prepared by the above procedure from 2 gm. (0.01 mole) of N-3-amino-4-methylphenyl-N′-nitroguanidine, was added to 50 cc. of acetone. A half gram of copper precipitated from copper sulphate solution with zinc was added and the suspension stirred for seven hours at room temperature. The insoluble portion was filtered off and washed with ether. This precipitate was refluxed with 50 cc. of ethanol and the copper removed by filtration. On evaporation of the

ethanol solution 0.7 gm. (32%) of N-3-chloro-4-methylphenyl-N′-nitroguanidine (m.p. 167–169°C.) was obtained. The melting point was raised to 170.5–172°C. after three crystallizations from ethanol. Calc. for $C_8H_9ClN_4O_2$: C, 42.03; H, 3.97; N, 24.52%. Found: C, 41.90; H, 4.40; N, 24.84%.

N-γ-Hydroxybutyl-N′-nitroguanidine

1-Amino-3-hydroxybutane (8.9 gm.; 0.1 mole) was dissolved in 50 cc. of water and 7.0 gm. (0.05 mole) of N-methyl-N-nitroso-N′-nitroguanidine was added portionwise while the temperature was held at 15–20°C. After gassing had ceased, the solution was evaporated to dryness, leaving 19.2 gm. of oil which still contained some water. A portion of the oil was nitrosated in the usual manner (3, 5, 6) to give a 50% yield of yellow crystalline nitroso compound melting at 99.5–100°C. with decomposition. Calc. for $C_5H_{11}N_5O_4$: C, 29.3; H, 5.33; N, 34.2%. Found: C, 29.01, H, 5.14; N, 33.92%.

In order to further identify this product, N-γ-hydroxybutyl-N-nitroso-N′-nitroguanidine (0.47 gm.; 0.003 mole) was nitrated as previously described (8) with nitric acid – acetic anhydride solution to give 0.4 gm. (70%) of N-γ-nitroxybutyl-N-nitroso-N′-nitroguanidine (m.p. 86–87°C. with decomposition). Three crystallizations from ethanol raised the melting point to 88–89°C. with decomposition. Calc. for $C_5H_{10}N_6O_6$: C, 24.00; H, 4.05; N, 33.55%. Found: C, 24.01; H, 4.14; N, 33.13%.

N-γ-Nitroxybutyl-N′-nitroguanidine

The oily N-γ-hydroxybutyl-N′-nitroguanidine (9.2 gm.) was nitrated with 10 mole equivalents of nitric acid in acetic anhydride at 5–10°C. for 45 min., and the product recovered in the usual manner (8). A yield of 51% based on the original N-methyl-N-nitroso-N′-nitroguanidine used in the preparation of N-γ-hydroxybutyl-N′-nitroguanidine was obtained. The actual yield from the nitration would be higher than this. The melting point of 123.5–126°C. of the crude was raised to 125–126°C. by crystallizing from water. Calc. for $C_5H_{11}N_5O_5$: C, 27.37; H, 5.01; N, 31.75%. Found: C, 27.38; H, 5.40; N, 31.66%.

1-*Nitro-2-amino-6-methyl-*Δ^2*-tetrahydropyrimidinium nitrate*

One gram (0.045 mole) of N-γ-nitroxybutyl-N′nitroguanidine was refluxed in water for one-half hour. The solution was evaporated to 1 cc. and 10 cc. of absolute ethanol was added. This procedure gave 0.72 gm. (72%) of product which gave a positive secondary nitramine test (1) and a positive test for nitrate ion using Nitron (10). The product melted at 115°C. Calc. for $C_5H_{11}N_5O_5$: C, 27.37; H, 5.01; N, 31.75%. Found: C, 27.04; H, 4.81; N, 31.05%.

A 0.35 gm. (0.0015 mole) sample of the cyclized product was dissolved in 2 cc. of water and a saturated aqueous picric acid solution added. A yellow crystalline picrate (0.6 gm.; 87%) separated immediately. The crude picrate melted at 166–173°C. This melting point was raised to 175–176°C. by two crystallizations from water. Calc. for $C_{11}H_{13}N_7O_9$: C, 34.01; H, 3.36; N, 25.35%. Found: C, 33.80; H, 3.18; N, 25.22%.

Acknowledgment

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THE ULTRAVIOLET ABSORPTION SPECTRA OF SEVERAL DEHYDRATION AND OXIDATION DERIVATIVES OF CHOLESTEROL[1]

By R. H. Cox[2, 3] and E. Y. Spencer

Abstract

Certain dehydration and oxidation products of cholesterol were prepared for subsequent animal experimentation. The ultraviolet absorption spectra of those derivatives possessing two or more conjugate chromophores were determined spectrophotometrically over the wave length range of 220 mμ to 350 mμ. The relative coincidence of the experimental λ_{max} and ϵ_{max} values of cholestadiene-3, 5 and of the enol acetate of cholesten–4–one–3 is offered as support for a 3, 5-diene structure for the latter steroid in particular and for enol esters of 4,5-unsaturated 3-ketosteroids in general.

Introduction

The ultraviolet absorption spectra of certain dehydration and oxidation derivatives of cholesterol were determined in preparation for fractionation studies on the heated egg yolk and heated cholesterol diets used by Altschul (1) to produce experimental arteriosclerosis and also sclerosis of muscle and nerve fiber in the rabbit. A particular attempt was made to locate maxima and minima in regions of low absorption which might aid in the positive identification of derivatives of cholesterol which might be formed during the heating of the experimental diets. The reliability of Beer's law in the ultraviolet was also investigated in connection with possible quantitative studies. The results of animal experimentation with several of the steroids considered in this investigation have been reported elsewhere (2, 6).

Experimental

Cholesterilene (Cholestadiene-3, 5) and Dicholesteryl Ether

Cholesterol was dehydrated by heating with an equal weight of anhydrous copper sulphate, according to the method of Mauthner and Suida (15), to give the intramolecular dehydration product, cholesterilene (cholestadiene-3, 5) in 66% yield. Repeated recrystallization of the crude material from absolute ethanol yielded colorless needles, m.p. 79–80° C., $[\alpha]_D$ − 89.7°, ϵ_{max} = 18.450 at 235 mμ. Two repetitions of this dehydration procedure resulted in yields of cholesterilene equivalent to 62% and 68% of the theoretical. The rest of the starting material was converted in every case to the intramolecular dehydration product, dicholesteryl ether. Purification of the ether by recrystallization from benzene–ethanol gave a colorless product melting from 196° to 199°C. $[\alpha]_D$ − 39.0°.

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[3] *This work was done during the tenure of a Life Insurance Medical Research Fund Student Fellowship.*

Cholesterol was also dehydrated using an equal weight of potassium hydrogen sulphate, as suggested by Montignie (18), and gave approximately the same ratio of cholesterilene to dicholesteryl ether as that obtained in the copper sulphate dehydration. However, when the weight of potassium hydrogen sulphate was five times that of cholesterol, the ratio of cholesterilene to dicholesteryl ether was reversed, which confirmed the earlier findings (18).

Cholesteryl Acetate

Cholesteryl acetate was prepared in quantitative yield by treating cholesterol with excess acetic anhydride under reflux. The ester, after repeated recrystallization from absolute ethanol, was obtained as crystalline needles, m.p. 115–116°C., $[\alpha]_D$ − 43.8°.

7-Ketocholesteryl Acetate

7-Ketocholesteryl acetate was prepared by chromic acid oxidation of cholesteryl acetate in acetic acid solution at 55°C., according to the procedure of Windaus, Lettre and Schenck (26). The faintly greenish oxidation product, melting from 154° to 156°C., was obtained in a yield of 28% of the theoretical. Several recrystallizations from ethanol – diethyl ether gave long, colorless needles melting at 158–159°C., $[\alpha]_D$ − 103.2°. A further small amount of crude product was obtained by extracting the mother liquor with diethyl ether after the addition of sodium chloride solution. Additional oxidation runs carried out as before gave yields of purified 7-ketocholesteryl acetate ranging from 25% to 32% of the theoretical.

Subsequently, it was found that lowering the reaction temperature from 55°C. to 40°C., together with reducing by 50% the quantity of distilled water employed in the original procedure to dissolve the oxidizing agent, raised the yield of 7-ketocholesteryl acetate to 35–40%, an increase of roughly 10% over that reported by Windaus *et al.* This compares with a yield of 33% recently reported by Fieser *et al.* (10) using a strictly anhydrous medium.

7-Ketocholesterol

7-Ketocholesterol acetate was saponified at room temperature by suspension in 80% methanol containing a slight excess of potassium carbonate for a period of five to six days. The crude 7-ketocholesterol, obtained in approximately quantitative yield, was recrystallized several times from diethyl ether–ethanol and was obtained as colorless needles melting at 169–170°C., $[\alpha]_D$ − 103.8°. ϵ_{max} = 14.450 at 236 mμ.

When saponification was carried out more rapidly by refluxing for several hours in 80% methanol containing a slight excess of either potassium carbonate or potassium hydroxide, lower yields of a crude, somewhat brownish product were obtained. Purification of this crude saponification product proved difficult and resulted in a further appreciable loss of material. The smaller yields

of a more crude 7-ketocholesterol obtained when more drastic conditions were employed is attributed to the general sensitivity to alkali of an α, β-unsaturated carbonyl system.

7-Ketocholesterilene (7-Ketocholestadiene-3, 5)

7-Ketocholesterilene was prepared from 7-ketocholesteryl acetate according to the method of Stavely and Bergmann (24) in 95% yield. Three recrystallizations of the reaction product from absolute ethanol gave needles melting at 111–112°C., $[\alpha]_D$ − 314°, ϵ_{max} = 23,300 at 278 mμ.

Epimeric 7-Hydroxycholesterols

7-Ketocholesterol was reduced according to the general method of Meerwein and Schmidt (16) using isopropanol as hydrogen donor and aluminum isopropylate as catalyst. The crude, crystalline reduction product, recovered in 65% of the theoretical yield, melted gradually from 130° to 150°C., a melting point range in agreement with that cited by Buser (5), and gave the expected Lifschutz (14) color reaction. No attempt was made to resolve the crude mixture of α and β epimers. Subsequent ultraviolet absorption studies on this product revealed a small selective absorption in the region of 235 mμ, indicating the presence of some unreduced 7-ketocholesterol. Similar incomplete reduction was subsequently observed by Spencer and Lambert (22) with the Meerwein procedure, but these workers eventually obtained almost quantitative conversion to the diol by employing a lithium aluminum hydride reduction procedure. These observations are in agreement with the recent work of Fieser *et al.* (10), who employed the latter reduction procedure but isolated the reaction product as the dibenzoate.

Cholestenone (Cholesten-4-one-3)

The general method of Oppenauer (19) was followed, but the more readily prepared aluminum isopropylate was substituted for aluminum tertiary butylate as catalyst for the reaction. The yield of almost colorless cholestenone, m.p. 77–79°C., was 76% of the theoretical. Repeated recrystallization from ethanol–diethyl ether gave material melting at 80-81°C., $[\alpha]_D$ + 88.0°, ϵ_{max} = 15,725 at 240 mμ.

In subsequent oxidations, additional modifications were introduced into the original Oppenauer procedure; in particular, these changes were designed to avoid the troublesome emulsions often encountered in the washing of the benzene extracts and to eliminate the time-consuming problem of bringing about crystallization of the oxidation product. After the oxidation step had been carried out according to the original instructions, the procedure was modified as follows.

Distilled water (slightly more than 3 moles per mole of catalyst) was added to the still warm contents of the reaction vessel to bring about hydrolysis of the aluminum alkoxide catalyst, and stirring was continued for an additional

30 min. The aluminum hydroxide gel so formed was removed from the cold reaction mixture by centrifuging, and the solution of sterol obtained by decantation was concentrated under vacuum (20 mm.) to a yellowish oil. This residue was dissolved in diethyl ether and the ethereal solution was washed with 10% sulphuric acid to remove remaining traces of aluminum hydroxide and then with distilled water until the washings were neutral to litmus. The ethereal solution was then dried over anhydrous sodium sulphate and concentrated under a stream of dry air; gentle rubbing of the oil precipitate of cholestenone as it formed brought about immediate crystallization. When practically all of the diethyl ether had been removed by evaporation, ethanol was added and the mixture was refrigerated for several hours. The almost colorless precipitate was collected by suction filtration and washed thoroughly with ice-cold ethanol to remove residual mesityl oxide. The product was then dried under vacuum to remove final traces of mesityl oxide and was recrystallized as before.

Cholestenone Enol Acetate

Cholestenone was treated with excess acetyl chloride under reflux for 10 hr. The temperature of reflux assisted in the rapid elimination of hydrogen chloride formed in the reaction and hence prevented the transformation of the enol ester into an enol chloride (20). The brownish needles which precipitated when the reaction solution was cooled were washed with cold ethanol and recrystallized several times from ethanol–diethyl ether to give long, colorless needles melting at 80-81°C., $[\alpha]_D -$ 92.0, ϵ_{max} = 19,600 at 235 mμ.

Melting Points

All melting points reported have been corrected against reliable standards.

Optical Rotation Measurements

All specific rotatory values cited for the above cholesterol derivatives were determined at room temperature, which varied from 20°C. to 25°C., with the Rudolph No. 127 precision polarimeter, using a macro-tube 2 dm. in length. Chloroform, purified by refluxing with and distilling from anhydrous calcium chloride, was used as the solvent. The concentrations of the steroids under investigation varied, but were usually of the order of 1–2%. For the sodium "D" line, the wave length used for all rotation measurements, monochromator and slit width settings gave an isolated band width of 9.5 mμ.

Ultraviolet Spectrophotometry

Spectral determinations were made over a wave length range of 220 mμ to 350 mμ with the Beckman DU quartz spectrophotometer. Density readings were never further apart than 4 mμ, while in regions where the optical density was changing rapidly the interval was reduced to 2 mμ, and in the immediate neighborhood of maxima and minima to 1 mμ. The absorption cells were of silica, each having a thickness of 1.001 cm. In every case, concentrations of

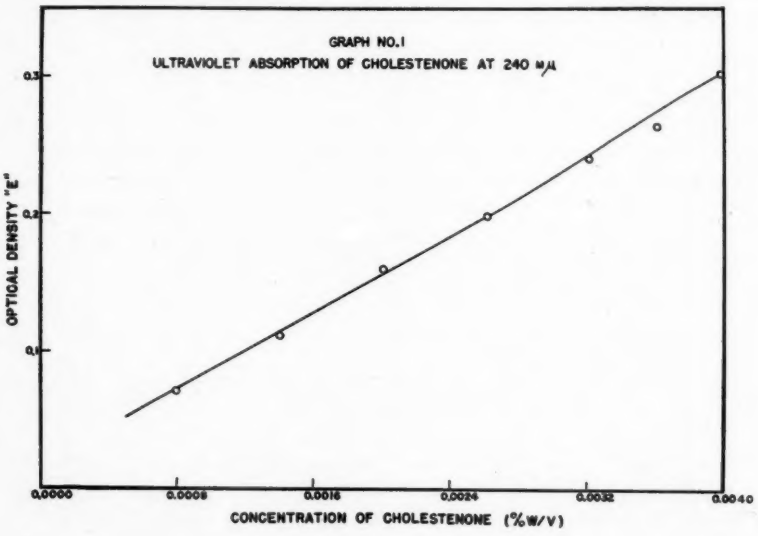
0.1%, 0.01%, and 0.001% of each compound were employed; the stronger concentrations were examined in regions of lower absorption in an attempt to find weak maxima and minima not discernible when concentrations low enough to permit total determination of the curve were used.

A commercial grade of absolute ethanol was found by spectral tests to be satisfactory as a solvent without special purification. In some cases it was necessary, because of the limited solubility in ethanol, to dissolve the sample in a small amount of diethyl ether and then dilute the ether solution to the desired volume with ethanol. The final concentration of ether was never greater than 2% and was of the order of 0.02% in the weakest (0.001%) steroid solutions. In all cases, the reference liquid used as a blank in the spectrophotometer was identical with the solvent of the solution under test, each component being taken from the same batch.

Ultraviolet Absorption Results and Discussion

Validity of Beer's Law

It now appears generally accepted that Beer's law always holds for dilute solutions and that the deviations sometimes encountered are due to a change in the absorbing species with concentration. Graph No. 1 shows that Beer's law is obeyed within the limits of experimental error with ethanolic solutions of cholestenone over the approximate concentration range of 0.001% to 0.005%. (Absorption values were measured at the λ_{max} 240 mμ). However, when the steroid concentration was increased by a further factor of 20 (i.e., to 0.1%), the absorption increased by only a fraction of this factor. This "falling-off" of the ϵ_{max} value which accompanies an increase in concentration was addition-



ally noted during spectral curve determinations, when concentrations of 0.001%, 0.01%, and 0.1% were employed with each steroid investigated. It was also noted that the extrapolated curve failed to pass through the origin.

Spectral Absorption Measurements

Experimental values of molecular extinction coefficients reported for all maxima and minima have been calculated from spectral absorption curves determined for the steroids under investigation. Where the order of magnitude was large ($\epsilon > 1000$), calculations were based upon absorption curves determined using concentrations of 0.001% steroid; on the other hand, where $\epsilon < 1000$, calculations were made from absorption values for solutions of 0.1% concentration. Although this policy introduces the variation of ϵ_{max} with concentration change seen experimentally, no adjustment for concentration effect was attempted because of the lesser importance, in a quantitative sense, of maxima and minima in regions of low absorption. The spectral band widths reported for individual maxima and minima were calculated from the observed slit-width and the calibration chart accompanying the spectrophotometer.

For comparison purposes, λ_{max} and ϵ_{max} values for the steroids under investigation have also been cited from the literature.

α, β-Unsaturated Ketones

It was early noted (3, 17) that α, β-unsaturated ketones have characteristic spectra. An isolated carbonyl group gives a low intensity band (ϵ = 10–100) near 275 mμ, while an ethylenic group gives a high intensity band ($\epsilon = 10^4$) near 195 mμ. When these two groups are conjugated, producing an α, β-unsaturated carbonyl compound, the two bands, referred to as K and R bands following Burawoy's (4) terminology, occur at longer wave lengths.

Band	Wave length	Intensity
K band	220-260 mμ	10^4
R band	310-330 mμ	10^2

Since the R band is of such low intensity, most attention is focused on the K band.

Cholestenone (Cholesten-4-one-3)

	Experimental values		Spectral band width (mμ)	Literature values		Ref.
	λ_{max} (mμ)	ϵ_{max}		λ_{max} (mμ)	ϵ_{max}	
Maximum	240	15,725	2.1	242	16,300	(13)
Plateau	312	120	2.9	312	76	(13)
				285	55	(13)

Cholestenone, having an α, β-unsaturated ketonic grouping in ring A was found to have a λ_{max} at 240 mμ (K band) and a plateau on the absorption curve at 312 mμ (suggestive of the R band). Good agreement is seen with available literature values.

7-Ketocholesterol

	Experimental values		Spectral band width (mμ)	Literature values		Ref.
	λ_{max} (mμ)	ϵ_{max}		λ_{max} (mμ)	ϵ_{max}	
Maxima	236	14,450	2.0	238	12,500	(27)
	329	50	3.3			
Minimum	284	20	2.4			

7-Ketocholesterol, having an α, β-unsaturated ketonic grouping in ring B, showed a strong selective absorption maximum at 236 mμ (K band) and a weak absorption maximum at 329 mμ (possibly the R band), the latter previously unreported. Fair agreement is seen between the values of λ_{max} and ϵ_{max} determined in this investigation for the K band, and those previously reported.

α, β: γ, δ-Diunsaturated Ketones

Acyclic triply conjugated systems involving two ethylenic and one ether group usually absorb maximally between 240 and 265 mμ, λ_{max} being little affected by the arrangement of the chromophores. The absorption of triple isocyclic systems is similar to that of the corresponding acyclic systems, with bands situated at somewhat higher wave lengths (about 20 mμ) than in analogous acyclic compounds, provided that all three chromophores are not in the same ring (9). Examples of three conjugated isocyclic chromophores are almost wholly confined to the steroid series.

7-Ketocholesterilene (7-Ketocholestadiene-3, 5)

	Experimental values		Spectral band width (mμ)	Literature values		Ref.
	λ_{max} (mμ)	ϵ_{max}		λ_{max} (mμ)	ϵ_{max}	
Maximum	278	23,300	2.2	280	23,300	(27)
Shoulder	336	150	3.4			

A comparison of the absorption spectrum of 7-ketocholesterilene with that of the α, β-unsaturated ketone 7-ketocholesterol readily illustrates the appreciable increase in λ_{max} and ϵ_{max} which accompanies the introduction of an additional conjugated unsaturation. The shoulder on the absorption curve seen at 336 mμ suggests that this may be the new position of the R band on addition of the third conjugated chromophere. The ϵ_{max} value for 7-ketocholesterilene determined at 278 mμ (K band) is in excellent agreement with the previous value reported in the literature. The fact that our experimental λ_{max} values

for 7-ketocholesterilene and for 7-ketocholesterol are 2 mμ lower than those reported by Wintersteiner and Bergstrom (27) suggests a linear difference of 2 mμ in the wave length calibrations of the respective spectrophotometers employed.

Conjugated Dienes

Cyclic conjugated dienes fall into two broad groups ,i.e., homoannular dienes, with the conjugated unsaturations in the same ring, which absorb in the region of 260–285 mμ and heteroannular dienes, with the conjugated unsaturations in different rings, which absorb in the region of 230–250 mμ (9). Both cholesterilene and the enol acetate of cholestenone are considered members of the latter group.

Cholesterilene (Cholestadiene–3, 5)

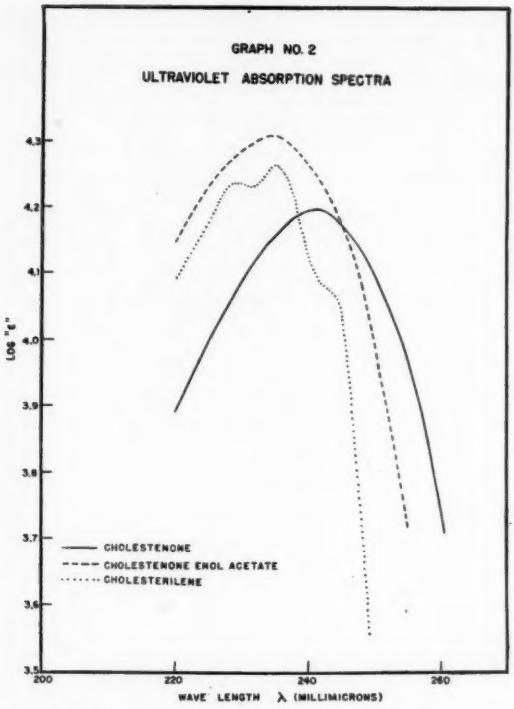
	Experimental values		Spectral band width (mμ)
	λ_{max} (mμ)	ϵ_{max}	
Maxima	235	18,450	2.0
	229	17,300	2.0
Minimum	231	17,000	2.0
Shoulder	243	12,050	2.1

Cholesterilene was originally assigned the structure of cholestadiene–2, 5 by Heilbron (11), but the high absorption of cholesterilene in the ultraviolet is now considered indicative of a conjugated system. The maxima reported at 249, 304, and 312 mμ in its absorption spectrum (12) were later not confirmed; instead, maxima at 229, 235, and 244 mμ (21, 23) and at 235 and 245 mμ (7) have been reported. In the present investigation, maxima were observed at 229 mμ, and at 235 mμ, and a shoulder was found on the curve at 243 mμ (see Graph No. 2); the experimentally determined ϵ_{max} values were of the same order of magnitude as those cited in the literature.

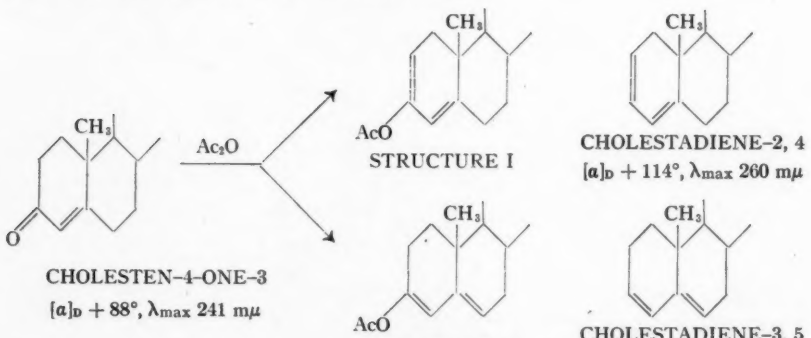
Cholestenone Enol Acetate (Cholesta–3, 5–dien–3yl Acetate)

	Experimental values		Spectral band width (mμ)
	λ_{max} (mμ)	ϵ_{max}	
Maximum	235	20,400	2.0
Plateaux	231	19,600	2.0
	312	130	2.9

In addition to the main absorption maximum at 235 mμ, transient plateaux were observed on the spectral curve for cholestenone enol acetate in the neighborhood of 231 mμ and 312 mμ. The ϵ_{max} value at 235 mμ is in agreement with that determined by Westphal (25), but the corresponding λ_{max} reported by this worker was that of 240 mμ.



Two possible structures have been proposed for 3-enol esters of 4, 5-unsaturated 3-ketosteroids in general, and hence for the enol acetate of cholestenone in particular. These are shown diagrammatically below, together with the structures and certain physical constants of the corresponding known cholestadienes and also of cholestenone itself.



CHOLESTEN-4-ONE-3
$[\alpha]_D + 88°$, λ_{max} 241 mμ

STRUCTURE I

STRUCTURE II

CHOLESTADIENE-2, 4
$[\alpha]_D + 114°$, λ_{max} 260 mμ

CHOLESTADIENE-3, 5
$[\alpha]_D - 90°$, λ_{max} 235 mμ

FIG. 1.

Since the specific optical rotation was seen to change from + 88° to − 92° (i.e., to approximately the same negative value as that determined for cholestadiene–3, 5),while the rotation of cholestadiene–2, 4, with a diene structure analogous to that of structure I, is markedly positive, Structure II is suggested for the enol acetate. Similar support has already been offered for Structure II by Westphal (25), based upon optical rotation values differing slightly in magnitude from those reported by us.

Wesphal further supported this postulated shift of the homoannular 2, 4–diene system to the heteroannular 3, 5–diene type by comparing the respective ultraviolet absorption spectra of these steroids. He interpreted the λ_{max} of the enol acetate at 240 mμ as that characteristic of a 3, 5–diene system, since cholestadiene–3, 5 absorbs maximally at 235 mμ and cholestadiene–2, 4 at 260 mμ. However, Westphal's λ_{max} at 240 mμ is somewhat higher than that (about 235 mμ) calculated empirically for this compound by Fieser (9) and others, and in addition is at the same wave length as that of the unesterified parent ketone cholestenone.

In the present investigation, a definite shift in the λ_{max} from 240 mμ to 235 mμ (i.e., to the λ_{max} of cholestadiene–3, 5) was observed upon acetylation of cholestenone. The strict coincidence of the λ_{max} value of cholestenone enol acetate with that of cholestadiene–3, 5, and hence with the empirically calculated value, is considered as supporting more strongly Westphal's postulate of a 3, 5–diene structure. (The authors, in the light of their experimental findings, question Woodward's (28) empirical calculations which predicted a λ_{max} for cholestenone enol acetate about 5 mμ higher than that for cholestadiene–3, 5.) The comparison of the absorption spectra of cholestenone, cholestenone enol acetate, and cholestadiene–3, 5 (cholesterilene) made in Graph No. 2 readily illustrates the shift in λ_{max} on acetylation and the coincidence of the λ_{max} values of the last two steroids. Good agreement is also seen between the λ_{max} values of the last two compounds.

Acknowledgments

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BIOCHEMISTRY OF THE USTILAGINALES

II. ISOLATION AND PARTIAL CHARACTERIZATION OF USTILAGIC ACID[1]

By R. U. Lemieux, J. A. Thorn, Carol Brice, and R. H. Haskins

Abstract

The needlelike crystals which form in aerated submerged cultures of the corn smut *Ustilago zeae* (PRL-119) were found to be a D-glucolipid, m.p. 146-7°C., $[\alpha]_D^{23} + 7°$ (pyridine), which contained one carboxyl group, two ester groups, at least two terminal methyl groups, and two D-glucose residues per mole. On the basis of elementary analyses, neutralization equivalent, and molecular weight estimations, the molecular formula of the material isolated was approximately $C_{37}H_{62-6}O_{17}$. The substance was not shown to be chemically homogeneous. Conditions used in culturing the fungus in stirred fermentors to produce the crystalline metabolic product, which was termed "ustilagic acid", are described, and a procedure for the isolation of the material is given.

Haskins (1) has shown that the corn smut *Ustilago zeae* (PRL-119) can dissimilate carbohydrates rapidly in aerated submerged cultures with the production of a substance which precipitated in the culture mixture in the form of long needlelike crystals. Since the culture mixture displayed pronounced antibiotic activity (1), our interest in the chemical nature of the substance was aroused.

The conditions used for the cultivation of the fungus and the production of the material investigated in this work are described in the experimental section. A culture medium containing D-glucose, urea, corn steep liquor, and mineral salts buffered by calcium carbonate was used. The fungus was grown successfully in 30-liter stainless steel fermentors.

Tests showed the crystalline material which was deposited in the culture medium to be readily soluble in methanol, pyridine, 2,3-butanediol, and 1,2-propanediol, only sparingly soluble in ethanol, butanol, and acetone, and insoluble in water, glycerol, ethyl acetate, diethyl ether, benzene, and petroleum ether. These remarkable solubility properties indicated that the substance was moderately polar in chemical nature and provided means for its separation from the other culture solids.

The isolation of the crystalline material was effected under the following mild conditions which would be expected in no way to alter the chemical nature of the substance. The culture solids were collected by filtration and pressed as dry as possible. The moist filter cake was extracted with methanol to remove the crystalline material and the methanolic extract was added to twice its volume of ether. The resulting solution, after clarification, was poured into double its

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volume of water. On standing overnight at 0°C., the mixture yielded a white flocculent precipitate which was collected by filtration and washed with water, then ether. The dried product varied in color from white to a light tinge of yellow. The yields of this crude product varied from 3 to 4.5 gm. per liter of culture medium.

In the above procedure, the ether was added to solubilize the substantial amounts of fatlike substances which were formed in the culture mixture. Although the crystalline material was insoluble in ether, a methanolic solution of the substance tolerated the addition of large amounts of ether without causing precipitation. This solubility property was found useful for the removal of the ash-producing substances which contaminated certain of the crude preparations. On the addition of water to a dilute ether–methanol solution of the substance, precipitation was slow and the product often was essentially crystalline. In many experiments, the portion of the product which precipitated in the ether phase appeared thoroughly crystalline and the crystalline samples used for some of the characterization work were isolated from this phase.

The material dissolved in 5% sodium bicarbonate solution to yield a soaplike solution. A sharp end point was observed on titration of a methanolic solution of the substance with aqueous alkali and phenolphthalein indicator. Purified samples exhibited neutralization equivalents of 777 ± 7. Since the material contained only carbon, hydrogen, and oxygen, it was concluded that it contained a carboxyl group. It is proposed that the acidic substance, isolated from cultures of *Ustilago zeae* in the manner described which showed the following physical and chemical properties, be designated as "ustilagic acid."

The molecular weight found for a crystalline sample of ustilagic acid was of the same magnitude as the neutralization equivalent. Elementary analyses thus indicated a molecular formula of approximately $C_{37}H_{62-6}O_{17}$. However, the substance was not shown to be chemically homogeneous.

The high oxygen content of ustilagic acid suggested the presence of carbohydrate residues, and methyl-α-D-glucopyranoside was readily isolated from the product of the methanolysis of the material. Estimation of the sugar content as D-glucose by the anthrone colorimetric method (3) showed the presence of sugar equivalent to two D-glucose residues per mole of ustilagic acid.

Ustilagic acid possessed alkali-labile groups, and treatment with excess 0.02 *N* sodium hydroxide at room temperature revealed a saponification equivalent one-half the neutralization equivalent. It was therefore apparent that ustilagic acid possessed two ester groups per mole. Terminal methyl group estimation by chromic oxide oxidation (2) indicated the presence of at least two CH_3–C groups per mole of ustilagic acid.

The 12 oxygen atoms which would reside in two D-glucose residues and the four oxygen atoms in the carboxyl and the two acyl groups accounted for all but one of the oxygen atoms in the approximate molecular formula. $C_{37}H_{62-6}O_{17}$.

It was thus apparent that the remainder of the substance was essentially hydrocarbon in chemical nature and that ustilagic acid was a D-glucolipid.

Crystalline ustilagic acid samples melted at temperatures which varied from 144° to 147°C. The crystalline sample herein reported melted at 146–147°C. and was weakly dextrorotatory, specific rotation +7°, in pyridine.

Experimental

Production of Ustilagic Acid

The ustilagic acid used throughout the present work was produced by the aerobic fermentation of cerelose by the corn-smut fungus *Ustilago zeae* (PRL-119) (1). The medium used contained, in tap water, 1.0 gm. potassium dihydrogen phosphate, 0.6 gm. corn steep liquor, 0.6 gm. urea, 50 gm. cerelose (D-glucose monohydrate) and 1.7 gm. calcium carbonate, per liter.

The fermentations were carried out in 9-liter stirred aerated bottles each containing 4 liters of broth, and in 30-liter stainless steel fermentors, similar to the apparatus described by Rivett, Johnson, and Peterson (5), each containing 15 liters of fermentation broth. The fermentors containing the culture medium were autoclaved for the minimum time necessary to ensure sterilization and then cooled to about 30°C. in a water bath. A sterilized slurry of the calcium carbonate in an aqueous solution of the urea was added aseptically to the cooled medium.

The culture medium was inoculated with 5% (volume per volume) of shake-flask culture mixture of the smut fungus grown for four days at 30°C. on a rotary shaker.

The fermenting culture mixtures were stirred in the range of 400–600 r.p.m. at 30°C., and aerated with approximately 0.27 liter of sterile air per liter of mixture per minute. Excessive foaming was controlled by the aseptic addition of sterile lard oil, about 20 ml. being required for each 15-liter fermentation. The fermentations in the 9-liter bottles were completed in five to six days, those in the 30-liter stainless steel fermentors in two to four days. The fermented broth was allowed to stand for 12 to 24 hours before isolation of ustilagic acid was begun.

Isolation of Ustilagic Acid

The culture solids were collected on a large Büchner funnel or with a laboratory model "Sparkler" filter press with 2% by weight of Johns-Manville Celite No. 545 as filter aid. The solids could also be collected conveniently in a basket centrifuge lined with cloth and a thin layer of shredded asbestos. In each case, the filter cake was pressed as dry as possible and stirred vigorously for about one hour with 250 ml. of methanol for each liter of fermentation broth filtered. The insoluble materials were collected by filtration and the filtrate was poured into twice its volume of diethyl ether. The small amount

of gelatinous precipitate which formed was removed by filtration and the filtrate was diluted with twice its volume of water. On standing overnight at 0°C., the mixture deposited a white flocculent precipitate, the material which we have termed "ustilagic acid". The substance was somewhat gelatinous and it was most conveniently collected by gravity filtration with hard filter paper. After washing with water, the material was triturated with ether several times. Usually, the composition of the dried product was essentially that of further purified samples. In some cases, however, the material contained appreciable ash largely derived from calcium. The salts responsible for this ash content were removed by dissolving the dry crude ustilagic acid in 20 parts of warm methanol and diluting the resulting solution with twice its volume of diethyl ether. The precipitate which formed immediately was removed by filtration and the ustilagic acid in the filtrate was reprecipitated by the addition of water in the manner described for the isolation of ustilagic acid.

The crude ustilagic acid samples were amorphous white to faintly yellow powdery solids. The purified preparations were colorless, both as solids or in methanolic solution. In many experiments, the portion of the ustilagic acid which had precipitated and remained in the ether phase of the precipitation medium was in the form of clusters of long needlelike crystals of the same appearance as the crystals observed in the culture mixture. The crystalline samples used for the characterization of ustilagic acid were obtained by the mechanical separation of this portion of the precipitate. Most successful fermentations yielded 3 to 4.5 gm. of crude ustilagic acid per liter of fermentation broth.

When the crude ustilagic acid showed a detectable ash content, the neutralization equivalent was higher but the saponification equivalent was essentially that of ash-free samples. This result indicated that the crude sample was composed of a mixture of free and neutralized ustilagic acid.

Partial Characterization of Ustilagic Acid

Table I lists the results of physical and chemical tests which are typical of those obtained for both crystalline and amorphous samples of ustilagic acid. Some of the analytical methods used are described below. The analytical results given in Table I are the average of duplicate estimations with the precision ordinarily expected for the various methods of analysis.

Neutralization and Saponification Equivalents of Ustilagic Acid

A weighed sample of ustilagic acid, about 35 mgm., was dissolved in 5 ml. of pure methanol in a 50 ml. glass-stoppered Erlenmeyer flask. The solution was cooled to near 0°C. in ice-water and slowly titrated to phenolphthalein end point with standard 0.01 *N* sodium hydroxide. The neutralization equivalent was calculated from the amount of alkali necessary to yield an indicator coloration which was stable for 15 sec. Exactly 10 ml. aliquots of 0.02 *N* sodium hydroxide was then added to the neutralized ustilagic acid solution and to 5 ml.

TABLE I

PARTIAL CHARACTERIZATION OF USTILAGIC ACID

	Ustilagic acid sample		Calc. for $C_{37}H_{62-6}O_{17}$ as $C_{20}H_{23-7}O(C_6H_{11}O_5)_2(COO)_2(CH_3)_2COOH$
No.	1	2	–
Physical state	Crystalline	Amorphous	–
Melting point	146–7°C.	–	–
$[\alpha]_D^{22}$ (*c*, 1 in pyridine)	+7°	+7.5°	–
Molecular weight	760	–	779–783
Neutralization equivalent	770	784	779–783
Saponification equivalent	384	392	389.5–391.5
% Glucose liberated on hydrolysis	42	48	46.0– 46.2
% Carbon	56.8	56.5	56.76– 57.04
% Hydrogen	8.2	8.5	8.04– 8.50
Terminal methyl as % acetyl	11.9	11.3	11.0–

of the methanol. The latter solution served as reagent blank. After standing for 24 hr. at room temperature, the alkali in each solution was determined by titration with 0.01 *N* hydrochloric acid. For accurate estimations the titration must not cause the precipitation of ustilagic acid, and a clear solution must result. This was facilitated by heating the solution to about 60°C. before titration. The difference between the blank titration and the titration of the ustilagic acid solution was taken as the volume of the standard hydrochloric acid solution equivalent to the ester groups in the sample.

The D-*Glucose Content of Ustilagic Acid*

Ustilagic acid, 400 mgm., was heated at 100°C. in a sealed tube with 10 ml. of 5% hydrogen chloride in methanol for 16 hr. The resulting solution was poured into 50 ml. of water and the precipitate which formed was removed by filtration. The chemical nature of this neutral precipitate will be described in the next paper of this series. It contained no carbohydrate, as shown by the anthrone test. The filtrate was neutralized with silver carbonate and the silver salts removed by filtration. The silver ions in the filtrate were removed as silver sulphide and the resulting aqueous solution was reduced *in vacuo* to a thick sirup which soon crystallized. The crystals were washed with ethanol and recrystallized from ethanol to yield 66 mgm. of material, m.p. 165–6°C., $[\alpha]_D^{20}$+ 158° (*c*, 0.8 in water), and this melting point was not depressed by admixture with authentic methyl-α-D-glucopyranoside, m.p. 165.5–166°C., $[\alpha]_D^{25}$+159° (*c*, 1 in water). A sample of the material was acetylated with acetic anhydride and sodium acetate to yield a derivative, m.p. 101–2°C., $[\alpha]_D^{22}$+ 131° (*c*, 0.9 in chloroform), which did not depress the melting point of methyl tetraacetyl-α-D-glucopyranoside, m.p. 101–2°C., $[\alpha]_D^{25}$+ 130.5° (*c*, 1 in chloroform). The yield of methyl-α-D-glucopyranoside was 33% of that expected if ustilagic acid contained two glucose residues per mole.

The isolation of methyl-α-D-glucopyranoside as a methanolysis product of ustilagic acid indicated that the substance was a glucoside and the colorimetric assay for sugar content by the anthrone method (3) was based on glucose. A weighed sample of ustilagic acid, 12 to 15 mgm., was dissolved in 5 ml. of 0.1 *N* sodium hydroxide solution in a 100 ml. volumetric flask. After dilution to 100 ml., 4 ml. aliquots of the solution were added to Coleman Model 6 spectrophotometer cuvettes. Eight milliliter volumes of the anthrone reagent (3) were then rapidly added to each cuvette by means of a syringe pipette and the color was allowed to develop for one-half hour. A standard absorption curve was established by treating solutions which contained known amounts of glucose, varying from 200 to 400γ per 4 ml. volume, in the same manner. The spectrophotometer was set at 100% transmittance with the solution resulting from the addition of 8 ml. of anthrone reagent to 4 ml. of water. The spectrophotometer readings were taken at λ540 and results were interpreted in the usual manner (3).

Determination of the Molecular Weight of Ustilagic Acid

We are indebted to Dr. I. E. Puddington, Division of Chemistry, National Research Council, for the molecular weight estimation on the crystalline ustilagic acid sample. The microtechnique for the accurate determination of molecular weights devised by Dr. Puddington (4) was used with methanol as solvent. The average value of several determinations was 760.

Acknowledgments

We are indebted to Dr. Ping Shu for valuable suggestions and help in establishing conditions for the 15-liter fermentations and to Mr. W. J. G. Cudmore who performed many of the larger scale fermentations and isolations.

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THE BIOCHEMISTRY OF THE USTILAGINALES

III. THE DEGRADATION PRODUCTS AND PROOF OF THE CHEMICAL HETEROGENEITY OF USTILAGIC ACID[1]

By R. U. Lemieux

Abstract

The D-glucolipid designated as ustilagic acid was shown to be a mixture of partially acylated derivatives of a di-D-glucosyl-dihydroxyhexadecanoic acid. The dihydroxyhexadecanoic acid was named "ustilic acid" and its di-D-glucosyl derivative was termed "glucoustilic acid". Alkaline hydrolysis of ustilagic acid yielded glucoustilic acid, acetic acid, *dextro*-β-hydroxy-*n*-caproic acid, *dextro*-β-hydroxy-*n*-caprylic acid and a small amount of *n*-caproic acid. The β-hydroxy-acids were characterized as *dextro*-β-hydroxy-*n*-caprohydrazide, m.p. 131–132°C., $[\alpha]_D$ + 15.9° (water), and *dextro*-β-hydroxy-*n*-caprylhydrazide, m.p. 127–128°C., $[\alpha]_D$ + 12° (water). Ustilic acid, m.p. 114–115°C., $[\alpha]_D$ − 6.3° (methanol), yielded a methyl ester, m.p. 80–81.8°C., $[\alpha]_D$ − 0.2 (chloroform). The methyl ustilate formed a di-phenylurethane derivative, m.p. 76–77°C. The infrared spectra of crystalline and amorphous samples of ustilagic acid are given. The specific rotation of glucoustilic acid, $[\alpha]_D$ − 11° (methanol) was that expected of β-D-glucosides and the infrared spectrum of this substance showed a marked resemblance to that of methyl-β-D-glucopyranoside.

In a previous publication (11) in this series, the production, isolation, and partial characterization of a D-glucolipid, termed ustilagic acid, was described. The material was found to contain one carboxyl group, two ester groups, at least two terminal methyl groups, and probably two D-glucose residues per mole (777 ± 7) and its composition was approximately $C_{37}H_{62-6}O_{17}$. The substance was isolated most readily as an amorphous solid although small amounts of crystalline preparations were obtained. The compositions of the crystalline and amorphous samples were essentially the same.

Forty-one grams of an amorphous preparation of ustilagic acid was saponified and the salts were decomposed by the addition of sulphuric acid. A highly swollen precipitate formed which was collected by filtration and thoroughly washed with cold water. On freeze drying, a white amorphous powder, 74% of the weight of the ustilagic acid hydrolyzed, was obtained. This water-insoluble material was a weak acid with neutralization equivalent 612; hence the yield obtained corresponded to one equivalent per mole of ustilagic acid hydrolyzed. The filtrate and wash waters from the isolation of the above water-insoluble acid were combined and subjected to steam distillation. A steam-volatile acid fraction was obtained which contained one equivalent of acidity per mole of ustilagic acid decomposed. The residue from the steam distillation was extracted with ether to yield an equivalent of steam-nonvolatile acid fraction per mole of ustilagic acid saponified. Thus ustilagic acid on alkaline hydrolysis gave both volatile and nonvolatile water-soluble acids and a water-insoluble acid.

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Methanolysis of the water-insoluble acid yielded 1.44 moles of D-glucose per neutralization equivalent. The D-glucose was isolated in the form of its methyl-α-D- and β-D-glucopyranosides and their acetyl derivatives. The glucosides were separated partially by fractional crystallization and partially by the precipitation of the β-anomer as its potassium acetate complex (14). A neutral, water-insoluble substance was also formed. This material was the methyl ester of a dihydroxyhexadecanoic acid. The acid was obtained in quantitative yield and it was designated "ustilic acid". The methyl ustilate, after several recrystallizations, melted at 80-81.8°C. However, other preparations of this material were obtained which melted at 84-85°C. Hydrolysis of the methyl ester yielded ustilic acid, m.p. 114-115°C., $[\alpha]_D$ −6.3° in methanol. The presence of two alcoholic hydroxyl groups in methyl ustilate was established by the preparation of a di-phenylurethane derivative, m.p. 76-77°C.

The estimation of the glucose content of the water-insoluble acid by the anthrone method (11, 13) showed it to contain two glucose residues per neutralization equivalent. The above described isolation of 1.44 moles of D-glucose per neutralization equivalent showed that the carbohydrate content was probably entirely derived from D-glucose. Elementary analyses of the substance yielded the carbon and hydrogen contents calculated for $(C_6H_{11}O_6)_2(C_{15}H_{29})$-COOH, the molecular formula expected from the nature of the hydrolytic products and the magnitude of the neutralization equivalent. It was thus evident that the water-insoluble acid was a di-D-glucosyl ustilic acid, and the material was given the trivial designation "glucoustilic acid".

The steam-volatile acid fraction was extracted with ether and converted to methyl ester with diazomethane. On solvent removal, most of the ester content was found in the ether distillate. The acid derived from this highly volatile ester displayed the Duclaux numbers of acetic acid, and the mixed melting point of its *p*-bromophenacyl ester with *p*-bromophenacyl acetate showed it to be acetic acid. A small amount of ester boiled between 144 and 154°C. This substance was shown to be methyl *n*-caproate, b.p. 150°C., by conversion to the *p*-bromophenacyl ester. The melting point of the latter substance was not depressed by admixture of *p*-bromophenacyl *n*-caproate. The amount of *n*-caproic acid thus isolated corresponded to only 0.06 mole per mole of ustilagic acid hydrolyzed.

The steam-nonvolatile acid fraction was isolated by extraction and methylated with diazomethane. After solvent removal, an oil remained which was separated into two components by fractional distillation under reduced pressure. The materials boiled at 194-197°C. and about 230°C. with decomposition at atmospheric pressure. Both components were dextrorotatory methyl esters which contained one hydroxyl and one terminal methyl group per saponification equivalent. The lower-boiling ester, $[\alpha]_D$ +28°, yielded a hydrazide, m.p. 131-132°C., $[\alpha]_D$ +15.9°, with the composition expected for $C_6H_{14}N_2O_2$. It was therefore apparent that the substance was derived from a monohydroxyhexanoic acid. The higher boiling ester, $[\alpha]_D$ + 24°, yielded a

hydrazide, m.p. 127-128°C., $[\alpha]_D$ + 12°. This melting point was depressed to 114-120°C. on admixture with the hydrazide derived from the lower-boiling methyl ester. The composition of the hydrazide corresponded to $C_8H_{18}O_2N_2$, and it was therefore apparent that the substance was derived from a monohydroxyoctanoic acid.

Oxidation of the monohydroxyhexanoic acid yielded *n*-butyric acid. It thus appeared that the substance was an optically active form of β-hydroxy-*n*-caproic acid. Levorotatory β-hydroxy-*n*-caprohydrazide, m.p. 130-131°C., $[\alpha]_D$ − 15.6°, obtained through the phytochemical reduction of β-keto-*n*-caproic acid (9), was shown to be the optical antipode of the hydrazide prepared from the monohydroxyhexanoic acid derived from ustilagic acid. The two hydrazides were mixed in equal amounts and the mixture was recrystallized to yield a substance, with a melting point 105-106°C., which remained unchanged on admixture of DL-β-hydroxy-*n*-caprohydrazide, m.p. 105.5-106°C.

The structure of the monohydroxyoctanoic acid was established in the same manner. The substance was assumed to be an optically active β-hydroxy-*n*-caprylic acid since, on oxidation, it yielded *n*-caproic acid. Levorotatory β-hydroxy-*n*-caprylhydrazide, m.p. 127.5-128°C., $[\alpha]_D$ − 11.4°, was prepared (9) from the β-hydroxy-*n*-caprylic acid obtained on the phytochemical reduction of β-keto-*n*-caprylic acid. The recrystallization of this hydrazide with an equal amount of the hydrazide of the monohydroxyoctanoic acid derived from ustilagic acid yielded a substance with a melting point 104-104.5°C. which remained unchanged on admixture of DL-β-hydroxy-*n*-caprylhydrazide, m.p. 104.2-104.7°C.

The infrared absorption spectra (see Fig. 1) obtained for both crystalline and amorphous samples of ustilagic acid were essentially the same. As would be expected from its chemical composition, ustilagic acid displayed strong absorption at the regions characteristic for carbonyl groups (5.76μ) and hydroxyl groups (2.92μ). Fig. 1 also shows the infrared spectrum obtained for the amorphous glucoustilic acid. This substance also showed absorption in the regions characteristic of the carbonyl and hydroxyl groups. The absorption in the carbonyl group region for glucoustilic acid was less intense, relative to that in the hydroxyl group region, than that found for ustilagic acid. This appears reasonable since glucoustilic acid was obtained by the deacylation of ustilagic acid. Kuhn (7) pointed out that acyl derivatives of sugars showed a strong absorption at 8 to 8.2μ which was characteristic of the ester groups. It is interesting to note that ustilagic acid showed a rather strong absorption band at 8.04 μ which was not present in the spectrum of glucoustilic acid.

The specific rotation of ustilic acid in methanol solution was weakly levorotatory, −6.3°, and that of glucoustilic acid was only slightly higher, −11°. These results can be interpreted (12, p. 375), on the basis of Hudson's Rules of Isorotation (6) to indicate that the D-glucosidic linkages in glucoustilic acid

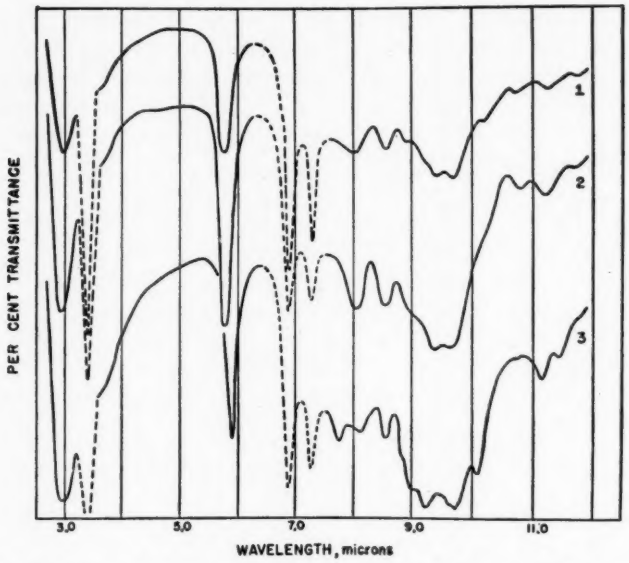
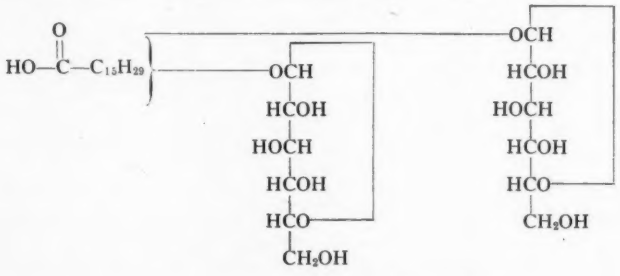


FIG. 1. *Infrared spectra. Curves 1 and 2—The crystalline and amorphous ustilagic acid samples, respectively, previously described (11). Curve 3—Amorphous glucoustilic acid.— — —Nujol absorption bands.*

were of the β-D-configuration. It is noteworthy in this respect that the infrared absorption spectrum of glucoustilic acid, Fig. 1, in the region 7.5 to 12.0 μ was nearly identical to the spectrum published by Kuhn (7) for methyl-β-D-glucopyranoside. This similarity of absorption spectra would not be surprising if glucoustilic acid possessed the structure shown below.



Glucoustilic acid (?)

This inference as to the structure of glucoustilic acid is being verified by conventional methods.

To summarize, the hydrolysis of an amorphous sample of ustilagic acid yielded a substance, designated glucoustilic acid, which, although it was amorphous, appeared to be chemically homogeneous. On this basis, glucousti-

lic acid was shown to be a di-β-D-glucosyl derivative of a dihydroxyhexadecanoic acid termed "ustilic acid". The further consideration of the structures of these substances is reserved for forthcoming publications in this series. Along with glucoustilic acid, the hydrolysis of ustilagic acid liberated four other acids, acetic acid, *dextro*-β-hydroxy-*n*-caproic acid, *dextro*-β-hydroxy-*n*-caprylic acid, and a small amount of *n*-caproic acid. Since ustilagic acid was found to contain only two ester groups per mole and a total of five different acids were identified as products of its alkaline hydrolysis, it was clear that the substance was a complex, the components of the mixture being made up of glucoustilic acid in combination with an average of two of the lower molecular weight acids per mole. The nature and distribution of the acyl groups in ustilagic acid will form the subject of a forthcoming publication in this series (8).

Experimental

Instrumentation

The infrared spectra were obtained by means of the National Technical Laboratories Model IR-2 infrared spectrometer. This instrument, thermostated at 25 ± 1°C., was calibrated to within 0.02 μ by means of the spectra of carbon dioxide, pyridine, and dioxane (15, p. 92). The transmissions through the sample and sample cell were compared with an empty cell. Nujol mulls (15, p. 93) of the materials were pressed between sodium chloride windows. The spectra represent a combination of the data obtained from recorder tracings and from point-by-point per cent transmission measurements.

All the rotations were determined in a 2 dm. polarimeter tube of about a 2 ml. capacity. The solutions were made up in a 2.5 ml. volumetric flask. All measurements were made at room temperature.

The melting points are uncorrected.

Alkaline Hydrolysis of Ustilagic Acid

Amorphous ustilagic acid, 41.0 gm., was dissolved in 400 ml. of methanol and the solution was refluxed for four hours with 352.2 ml. of 0.577 *N* sodium hydroxide solution. Neutralization of the saponified mixture required 103.8 ml. of 0.484 *N* sulphuric acid solution. Therefore, 153 m.e. of alkali was consumed. The salts of the organic acids were decomposed by the addition of 325 ml. of 0.484 *N* sulphuric acid solution and the mixture was kept at 0°C. for five hours. The heavy deposit of a highly swollen precipitate was collected by filtration on a large Büchner funnel. The material was washed with cold water then stirred with about 500 ml. of water and transferred back to the filter where it was pressed as dry as possible. This procedure was repeated three times more. The filter cake was pressed free of loosely held water, then frozen and dried by lyophilization. The product, after further drying *in vacuo* over calcium chloride for three days, weighed 30.5 gm. The fine, white amorphous powder, designated as glucoustilic acid, was soluble in alkali,

methanol, and pyridine, very sparingly soluble in water or ethanol, and insoluble in ether, chloroform, ethyl acetate, and benzene. Calc. for $(C_6H_{11}O_6)_2$ $C_{15}H_{29}COOH$: C, 54.89; H, 8.56; glucose, 58.7%; neutralization equiv., 612.7. Found: C, 54.61; H, 8.57; glucose, 58.5, 62.0%; neutralization equiv., 610, 612 (11). Oxidation in 30% chromium trioxide (10) yielded no steam-volatile acid. The yield of this substance, glucoustilic acid, $[\alpha]_D$ − 11° (*c*, 0.75 in methanol), $[\alpha]_D$ − 8.9° (*c*, 0.82 in 0.1 *N* sodium hydroxide), corresponded to 49.8 m.e. of acid.

The filtrate and wash waters from the above isolation were combined and concentrated by distillation at atmospheric pressure to about 500 ml. and the steam-volatile acids which remained in the residue were removed by continuing the distillation with steam. The distillation was stopped after steam-volatile acidity equivalent to 94.2 ml. of 0.577 *N* sodium hydroxide, 54.3 m.e., had passed into the distillate. At this point the vapors contained only a trace of acidity. The salts of the steam volatile acids were isolated by evaporation of the neutralized distillate.

Of the 153 m.e. of free or potential acidity in the ustilagic acid sample, 49.8 m.e. was isolated as glucoustilic acid and 54.3 m.e. was found to be derived from steam-volatile acid. The 48.9 m.e. of acid unaccounted for was in the residue from the steam distillation. This was shown by a separate experiment. Ustilagic acid, 1.94 gm., consumed 7.28 m.e. of base. Acidification and steam distillation of the filtrate from the removal of the glucoustilic acid precipitate yielded 2.42 m.e. of steam-volatile acid, and ether extraction of the residue yielded 2.32 m.e. of steam-nonvolatile, water-soluble, ether-extractable acid. This acid fraction was isolated from the residue of the steam distillation in the main experiment by continuous extraction with ether for 18 hr.

Methanolysis of Glucoustilic Acid

Glucoustilic acid, 20.0 gm., was heated under reflux with 250 ml. of 5% methanolic hydrogen chloride for 19 hr. The reaction mixture was poured into ice-water and the aqueous mixture was extracted four times with chloroform. The combined chloroform extracts were washed twice with water. The water extracts were combined and neutralized with 35 gm. of silver carbonate. The insoluble silver salts were removed by filtration and the dissolved silver ions were precipitated with hydrogen sulphide. The clear, colorless filtrate was evaporated *in vacuo* to a thick sirup which soon began to crystallize. The dry material was dissolved in 50 ml. of boiling methanol and the solution, on standing in the cold, deposited 5.33 gm. of a crystalline material, m.p. 165–166° C., $[\alpha]_D$ + 158° (*c*, 0.8 in water). The melting point was not depressed on admixture with an authentic sample of methyl-α-D-glucopyranoside, m.p. 165.5–166°C., $[\alpha]_D$ + 159° (*c*, 1 in water). The mother liquors were concentrated at room temperature to 15 ml. and this solution on cooling deposited 2.9 gm. of crystals, m.p. 137–150°C., which were recrystallized from 10 ml. of

methanol to yield 1.25 gm. of methyl-α-D-glucopyranoside, m.p. 165–166°C., $[\alpha]_D$ + 156° (*c*, 1.2 in water). The mother liquors from the above crystallizations were combined and evaporated to dryness. The dry sirup was dissolved in 11 ml. of dry ethanol, and 4 gm. of potassium acetate in 22 ml. of dry ethanol was added. The crystalline potassium acetate complex (14) which precipitated was washed twice with ethanol, twice with acetone, and dried. The yield was 3.79 gm. of a substance with m.p. 175–178°C., and $[\alpha]_D$ − 11.1° (*c*, 0.8 in water). The material was suspended in 30 ml. of acetic anhydride and heated on the steam bath for two hours. The reaction mixture was poured into crushed ice, and the mixture thoroughly stirred and left overnight at 4°C. The aqueous mixture was extracted with chloroform three times, the combined chloroform extracts were washed with water and dried, and the chloroform was removed by aeration. The resulting semicrystalline sirup was dissolved in a little hot ethanol and the solution was allowed to stand overnight in the cold. There was deposited 3.87 gm. of a white crystalline material, m.p. 104–105°C., $[\alpha]_D$ − 18° (*c*, 1.2 in chloroform). The melting point was not depressed by admixture with authentic methyl tetraacetyl-β-D-glucopyranoside. The mother liquors from the isolation of the potassium acetate complex were dried *in vacuo* to a semicrystalline sirup. This material was heated on the steam bath for two hours with 20 ml. of acetic anhydride. The sirupy acetyl derivative was isolated as described above. The dry yellow sirup was dissolved in a little ethanol and the solution was seeded with a few small crystals of methyl tetraacetyl-α-D-glucopyranoside. After standing overnight at 4°C., the crystals which had deposited were gathered by filtration and washed with ethanol. The yield was 1.00 gm. of material, m.p. 93–100°C. Recrystallization from ether–Skellysolve C mixture yielded 0.86 gm. of a substance, m.p. 101–101.5°C., $[\alpha]_D$ + 132° (*c*, 0.74 in chloroform), the melting point of which was not depressed by admixture of methyl tetraacetyl-α-D-glucopyranoside, m.p. 101–102°C. There was thus isolated from 20 gm. of glucoustilic acid 8.45 gm. of D-glucose in the form of essentially pure derivatives or 1.44 moles of the sugar per mole of glucoustilic acid.

The chloroform extract of the methanolysis mixture was dried over sodium sulphate and concentrated on the steam bath to a yellow oil which crystallized on cooling. The crystalline mass was ground to powder and thoroughly dried *in vacuo*. The yield was 9.8 gm. and the crude material melted at 79–81°C. Several recrystallizations from methanol did not raise the melting point above 80–81.8°C., $[\alpha]_D$ − 0.2 in chloroform. Calc. for $C_{17}H_{34}O_4$: C, 67.53; H, 11.33%; mol. wt., 302.4; sapon. equiv., 302.4. Found: C, 67.50; H, 11.47%; mol. wt. (Rast), 328; sapon. equiv., 300, 304. The yield of crude methyl ustilate was nearly quantitative.

Saponification of methyl ustilate and decomposition of the salt gave ustilic acid. After recrystallization from dioxane followed by two recrystallizations from 75% ethanol, the free acid melted at 114–115°C. with $[\alpha]_D$ − 6.3° (*c*, 1.1 in methanol). Calc. for $C_{16}H_{32}O_4$: C, 66.64; H, 11.19%; neutralization equiv., 288.4. Found: C, 66.52; H, 11.22%; neutralization equiv., 288, 289.

Methyl ustilate, 150 mgm., was heated at 100°C. with about 200 mgm. of phenylisocyanate and a drop of pyridine for 15 min. On cooling, the solution hardened to a crystalline mass which was washed with Skellysolve C. The material was treated with boiling Skellysolve C and the insoluble substances were removed by filtration. On cooling the filtrate, an oil separated which soon crystallized. After recrystallization from ether – Skellysolve C, the material melted at 76–77°C. Calc. for $C_{31}H_{44}O_6N_2$: C, 68.85; H, 8.20; N, 5.18%. Found: C, 68.95; H, 8.28; N, 5.15, 5.13%.

Characterization of the Steam-volatile Acids

The sodium salts of the steam-volatile acids (see above) were dissolved in 125 ml. of 0.5 *N* sulphuric acid and the solution was extracted continuously with ether for 24 hr. The ether extract was dried over sodium sulphate and treated with an excess of an ethereal solution of diazomethane. The ether was removed by rather rapid distillation through a Widmer fractionating column. Only a small amount of a yellow oil boiled above 100°C. Nearly all of this liquid distilled between 144° and 154°C. to yield 0.34 gm. of a methyl ester. The ester was saponified to yield a sodium salt which was treated with an alcoholic solution of *p*-bromophenacyl bromide. The product after two recrystallizations from ethanol melted at 70–71°C. and this melting point was unchanged on mixing with an authentic sample of *p*-bromophenacyl *n*-caproate, m.p. 71–72°C. Methyl *n*-caproate boils at 150°C. The ether distillate was refluxed for six hours with 60 ml. of 0.6 *N* sodium hydroxide. Titration of the resulting mixture showed that nearly all of the alkali was neutralized. The neutral solution was evaporated to dryness and the salts were dissolved in 75 ml. of 0.5 *N* sulphuric acid. The resulting solution was steam distilled until all volatile acids were recovered in the distillate. The first 115 ml. of distillate was kept for the determination of Duclaux numbers. Titration of a 10 ml. aliquot of this fraction required 17.50 ml. of 0.1 *N* sodium hydroxide. A 100 ml. aliquot was then distilled in the standard manner for the determination of Duclaux numbers (16, p. 120). The Duclaux numbers listed (16, p. 121) for acetic acid are 6.8, 7.1, and 7.4 and the corresponding numbers found were 6.0, 6.9, and 7.3. The combined distillates were neutralized and evaporated to the sodium salt. Treatment of the salt with *p*-bromophenacyl bromide yielded a *p*-bromophenacyl ester which after one recrystallization from ethanol melted at 84–85°C. The mixed melting point with an authentic sample of *p*-bromophenacyl acetate, m.p. 84–85°C., was 84–85°C.

Separation of the Steam-nonvolatile Acids.

The ether extract of the water-soluble, steam-nonvolatile acid fraction (see above) was dried over sodium sulphate and treated with an excess of diazomethane. The ether was removed by distillation to yield 6.5 gm. of a neutral yellow oil, sapon. equiv. 156, n_D^{25} 1.429. Fractional distillation (1) at 1 mm. pressure of 5.04 gm. of the material separated it into two components. The lower-boiling ester, sapon. equiv. 146, $[\alpha]_D$ + 28° (*c*, 0.86 in chloroform), n_D^{25} 1.427, boiled at 194–197°C. at 710 mm. in the Emich (3) micro boiling point determination and comprised 60–70% of the mixture. The higher

boiling ester, sapon. equiv. 168, $[\alpha]_D$ + 24° (*c*, 0.85 in chloroform), n_D^{25} 1.433, boiled (3) at about 230°C. with decomposition at 710 mm. Analysis showed each component to contain one hydroxyl group and one terminal methyl group (10) per saponification equivalent. The magnitude of the boiling points found for these substances required their molecular weights to be equal to their saponification equivalents. The proofs of the structures of the two esters are described separately below.

Characterization of dextro-Methyl β-hydroxy-n-caproate

The lower-boiling ester (see above), 0.292 gm., was dissolved in 12 ml. of water which contained 1.2 gm. of sodium hydroxide. To the solution, cooled in ice-water, was added dropwise 1.62 gm. of bromine. After standing at room temperature overnight, the solution was heated at 100°C. for 15 min., acidified with sulphuric acid and decolorized with sodium bisulphite. The solution was steam distilled to yield 150 ml. of distillate, which was treated with an excess of silver sulphate, and the volatile acids were removed by steam distillation. The distillate was neutralized and evaporated to the sodium salts, 2.2 m.e. The salts were decomposed by the addition of hydrochloric acid and portions of the resulting solution were subjected to partition chromatography under the conditions to be described in a forthcoming publication (8). The first acid found in the percolate appeared at the position characteristic of *n*-butyric acid. The zones for this acid from five such chromatograms were combined, neutralized and the resulting solution was evaporated to dryness to yield 0.18 m.e. of sodium salt. The salt in 2 ml. of water was heated with 50 mgm. of *p*-phenylphenacyl bromide in 3 ml. of ethanol for two hours. The substance which crystallized on cooling melted at 81.5–82°C. after one recrystallization from ethanol. This melting point was unchanged on admixture of an authentic sample of *p*-phenylphenacyl *n*-butyrate, m.p. 81.5–82°C.

The methyl ester, 381 mgm., was heated on the steam bath with 0.38 ml. of hydrazine hydrate for a half hour. Ethanol, 0.5 ml., was then added and the solution was refluxed for two hours. On cooling, the solution deposited crystals which were washed with cold ethanol. The yield was 312 mgm. of material, m.p. 129–131°C. Recrystallization from ethanol yielded pure material, m.p. 131–132°C., $[\alpha]_D$ + 15.9° (*c*, 0.75 in water). Calc. for $C_6H_{14}N_2O_2$: C, 49.29; H, 9.65%. Found: C, 49.25, 49.13; H, 9.89, 9.77%.

α, β-Hexenoic acid (2), 5 gm., was heated with boiling 10% aqueous alkali, 350 ml., for 33 hr. The resulting solution was treated with an excess of hydrochloric acid and freed of steam-volatile acids by steam distillation. The residue was extracted with ether to remove the steam-nonvolatile β-hydroxy-*n*-caproic acid (5). The ether extract was treated with an excess of diazomethane, and 1.65 gm. of methyl DL-β-hydroxy-*n*-caproate was obtained by distillation. The substance yielded a hydrazide, m.p. 105.5–106°C. which was purified by recrystallization from ethanol. Calc. for $C_6H_{14}O_2N_2$: C, 49.29; H, 9.65%. Found: C, 49.20, 49.14; H, 9.51, 9.68%.

The above described hydrazide, m.p. 131–132°C., $[\alpha]_D$ + 15.9°, obtained from the monohydroxyhexanoic acid derived from ustilagic acid, 10.65 mgm.,

was mixed with 10.65 mgm. of levorotatory hydrazide of β-hydroxy-*n*-caproic acid, m.p. 130–131°C., $[\alpha]_D$ − 15.6° (*c*, 0.9 in water) (9). The mixture was dissolved in the minimum amount of boiling ethanol and the crystalline material which separated on cooling was collected by filtration and dried. The yield was 18.6 mgm. or 87% of a substance, m.p. 105–106°C., $[\alpha]_D$ 0° (*c*, 0.5 in water). This melting point was unchanged on admixture of the above described DL-β-hydroxy-*n*-caprohydrazide.

The optically active β-hydroxy-*n*-caproic acid derived from ustilagic acid was not isolated in pure form; however, a chloroform solution of the substance was dextrorotatory, $[\alpha]_D$ + 30° (*c*, 2 in chloroform).

Characterization of dextro-Methyl β-Hydroxy-n-caprylate

The higher-boiling ester (see above), 0.336 gm., was dissolved in 12 ml. of water and 1.2 gm. of sodium hydroxide. Bromine, 1.62 gm., was added dropwise to the ice-cold solution. After standing at room temperature for four and one-half hours, the mixture was heated for 15 min. on the steam bath. The sodium salts of the steam volatile acids formed under these conditions were isolated as described above for the lower-boiling ester. The salts, 1.3 m.e., were dissolved in 1.5 ml. of *N* hydrochloric acid and the solution was diluted to 5 ml. The solution was turbid and possessed a strong odor similar to that of *n*-caproic acid. Partition chromatography (8) of 0.5 ml. aliquots of the mixture showed it to contain an acid fraction which passed through the chromatographic column almost as rapidly as the developing phase. Several chromatograms yielded 0.38 m.e. of this acid fraction which was converted to the sodium salt. The salt was treated with 103 mgm. of *p*-phenylphenacyl bromide in aqueous ethanol to yield an ester which melted at 61–63°C. after two recrystallizations from ethanol. This material with *p*-phenylphenacyl *n*-caproate, m.p. 65°C., yielded a mixture which melted at 62–65°C.

The ester, 407 mgm., was heated on the steam bath for a half hour with 0.41 ml. of hydrazine hydrate. Ethanol, 0.5 ml., was added and the solution was refluxed for two hours. On cooling, the solution deposited a precipitate which, after washing with ethanol and drying, weighed 248 mgm. and melted at 124-127°C. Recrystallization from ethanol yielded pure material, m.p. 127–128°C., $[\alpha]_D$ + 12° (*c*, 1.1 in water). Calc. for $C_8H_{18}O_2N_2$: C, 55.16; H, 10.41%. Found: C, 54.80, 54.74; H, 10.32, 10.43%. The melting point was depressed to 114–120°C. on admixture of the hydrazide derived from the lower-boiling ester.

Ethyl β-keto-*n*-caprylate (17), 10 gm., prepared from *n*-caproyl chloride and ethyl acetoacetate under conditions which paralleled the preparation of ethyl β-keto-*n*-caproate (4), was dissolved in 25 ml. of ethanol. The solution was cooled to about 0°C. and 127 ml. of 0.456 *N* potassium hydroxide solution was added dropwise with vigorous stirring. The stirring was continued for two hours and the solution was left at room temperature overnight. To the solution, cooled in ice-water, powdered 3% sodium amalgam, 150 gm., was added, about 5 gm. at a time, with vigorous mechanical stirring. The pH of the mixture was kept between 7 and 9 by the addition of 0.1 *N* hydrochloric

acid. After the sodium appeared completely reacted, the aqueous phase, clarified by filtration, was concentrated *in vacuo* to about 100 ml. and made strongly acid by the addition of 25 ml. of concentrated hydrochloric acid. The unreacted β-keto-*n*-caprylic acid which separated as an oil soon crystallized on cooling to 0°C. and it was removed by filtration. The clear filtrate was extracted continuously with ether for 18 hr. The ether extract, after drying over sodium sulphate, was treated with an excess of diazomethane. On solvent removal an oil remained which on distillation yielded 1.38 gm. of methyl DL-β-hydroxy-*n*-caprylate, b.p. 110–112°C. at 9 mm., n_D^{25} 1.432. The ester was treated with hydrazine hydrate in the usual manner to yield the hydrazide, m.p. 104.2–104.7°C., which was purified by recrystallization from ethanol. Calc. for $C_8H_{18}O_2N_2$: C, 55.16; H, 10.41%. Found: C, 55.47; H, 10.25%.

The above hydrazide, m.p. 127–128°C., $[\alpha]_D$ + 12°, prepared from the monohydroxyoctanoic acid derived from ustilagic acid, 11.60 mgm., was mixed with 11.65 mgm. of the hydrazide of β-hydroxy-*n*-caprylic acid (9), m.p. 127.5–128°C., $[\alpha]_D$ − 11.4°. The mixture was dissolved in a few drops of boiling ethanol and, on cooling, 14.8 mgm., 64% yield, of crystals was deposited, m.p. 104–104.5°C., $[\alpha]_D$ 0° (*c*, 0.4 in water). The melting point of this substance was unchanged by admixture of the above described DL-β-hydroxy-*n*-caprylhydrazide.

The optically active β-hydroxy-*n*-caprylic acid derived from ustilagic acid was not isolated in the pure form. That the substance is dextrorotatory was shown by the fact that its optical antipode (9) was levorotatory, $[\alpha]_D$ − 21° (*c*, 1.9 in chloroform).

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